02 INFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS

Submit only ONE copy of this form **for each PI/PD and co-PI/PD** identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.B. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Mark Fishbein

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)

- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)

- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☐

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☐

Ethnicity Definition:
Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.
Race Definitions:
American Indian or Alaska Native. A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment.
Asian. A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
Black or African American. A person having origins in any of the black racial groups of Africa.
Native Hawaiian or Other Pacific Islander. A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
White. A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

WHY THIS INFORMATION IS BEING REQUESTED:

The Federal Government has a continuing commitment to monitor the operation of its review and award processes to identify and address any inequities based on gender, race, ethnicity, or disability of its proposed PIs/PDs. To gather information needed for this important task, the proposer should submit a single copy of this form for each identified PI/PD with each proposal. Submission of the requested information is voluntary and will not affect the organization's eligibility for an award. However, information not submitted will seriously undermine the statistical validity, and therefore the usefulness, of information recieved from others. Any individual not wishing to submit some or all the information should check the box provided for this purpose. (The exceptions are the PI/PD name and the information about prior Federal support, the last question above.)

Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational oppurtunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

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PI/PD Name: Steven B Broyles

Gender: ☒ Male ☐ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race:
(Select one or more)
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status:
(Select one or more)
- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☐

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☒

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Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational oppurtunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

List of Suggested Reviewers or Reviewers Not To Include (optional)

SUGGESTED REVIEWERS:

Michael L. Arnold
John M. Burke
Diane R. Campbell
Mitchell B. Cruzan
Scott A. Hodges
Steven D. Johnson
Susan R. Kephart
Loren H. Rieseberg
Douglas W. Schemske
Joseph H. Williams
Lorne M. Wolfe

REVIEWERS NOT TO INCLUDE:

Not Listed

List of Suggested Reviewers or Reviewers Not To Include (optional)

SUGGESTED REVIEWERS:
Not Listed

REVIEWERS NOT TO INCLUDE:
Not Listed

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 04-2 NSF 04-2	FOR NSF USE ONLY NSF PROPOSAL NUMBER **0415895**
FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.) DEB - Population Biology	

DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION
				075461814	

EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN)	SHOW PREVIOUS AWARD NO. IF THIS IS	IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY?
646000819	☐ A RENEWAL ☐ AN ACCOMPLISHMENT-BASED RENEWAL	YES ☐ NO ☒ IF YES, LIST ACRONYM(S)

NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE Mississippi State University	ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE Mississippi State University Mississippi State, MS. 39762
AWARDEE ORGANIZATION CODE (IF KNOWN) 0024232000	
NAME OF PERFORMING ORGANIZATION, IF DIFFERENT FROM ABOVE	ADDRESS OF PERFORMING ORGANIZATION, IF DIFFERENT, INCLUDING 9 DIGIT ZIP CODE
PERFORMING ORGANIZATION CODE (IF KNOWN)	

IS AWARDEE ORGANIZATION (Check All That Apply) (See GPG II.C For Definitions)
☐ SMALL BUSINESS ☐ MINORITY BUSINESS
☐ FOR-PROFIT ORGANIZATION ☐ WOMAN-OWNED BUSINESS
☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT **Floral Scents of Hybrids: Bridge or Barrier to Interspecific Gene Flow?**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ 396,926	36 months	06/01/04	

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

- ☒ BEGINNING INVESTIGATOR (GPG I.A)
- ☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C)
- ☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG I.B, II.C.1.d)
- ☐ HISTORIC PLACES (GPG II.C.2.j)
- ☐ SMALL GRANT FOR EXPLOR. RESEARCH (SGER) (GPG II.D.1)
- ☐ VERTEBRATE ANIMALS (GPG II.D.5) IACUC App. Date ________
- ☐ HUMAN SUBJECTS (GPG II.D.6) Exemption Subsection ______ or IRB App. Date ______
- ☐ INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES INVOLVED (GPG II.C.2.j) ________
- ☐ HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.E.1)

PI/PD DEPARTMENT Department of Biological Sciences	PI/PD POSTAL ADDRESS Mississippi State University P. O. Box GY Mississippi State, MS 39762 United States
PI/PD FAX NUMBER 662-325-7939	

NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Electronic Mail Address
PI/PD NAME Mark Fishbein	PhD	1996	662-325-7577	fish@biology.msstate.edu
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				

Electronic Signature

CERTIFICATION PAGE

Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPG), NSF 04-2. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U. S. Code, Title 18, Section 1001).

In addition, if the applicant institution employs more than fifty persons, the authorized official of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of Grant Policy Manual Section 510; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution's expenditure of any funds under the award, in accordance with the institution's conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Drug Free Work Place Certification

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Appendix C of the Grant Proposal Guide.

Debarment and Suspension Certification

(If answer "yes", please provide explanation.)

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency? Yes ☐ No ☒

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Debarment and Suspension Certification contained in Appendix D of the Grant Proposal Guide.

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, ''Disclosure of Lobbying Activities,'' in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME **Lynda G Tuck**	**Electronic Signature**	**Jan 9 2004 5:22PM**

TELEPHONE NUMBER	ELECTRONIC MAIL ADDRESS	FAX NUMBER
662-325-7404	**lynda@spa.msstate.edu**	**662-325-3803**

*SUBMISSION OF SOCIAL SECURITY NUMBERS IS VOLUNTARY AND WILL NOT AFFECT THE ORGANIZATION'S ELIGIBILITY FOR AN AWARD. HOWEVER, THEY ARE AN INTEGRAL PART OF THE INFORMATION SYSTEM AND ASSIST IN PROCESSING THE PROPOSAL. SSN SOLICITED UNDER NSF ACT OF 1950, AS AMENDED.

0415895

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 04-2

NSF 04-2

FOR NSF USE ONLY

NSF PROPOSAL NUMBER

0415358

FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)

DEB - Population Biology

DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION
				188731012	

EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN)

146013200

SHOW PREVIOUS AWARD NO. IF THIS IS
☐ A RENEWAL
☐ AN ACCOMPLISHMENT-BASED RENEWAL

IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? YES ☐ NO ☒ IF YES, LIST ACRONYM(S)

NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE

SUNY College at Cortland

ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE

SUNY College at Cortland
P. O. Box 9
Albany, NY. 122010009

AWARDEE ORGANIZATION CODE (IF KNOWN)

0028431000

NAME OF PERFORMING ORGANIZATION, IF DIFFERENT FROM ABOVE

ADDRESS OF PERFORMING ORGANIZATION, IF DIFFERENT, INCLUDING 9 DIGIT ZIP CODE

PERFORMING ORGANIZATION CODE (IF KNOWN)

IS AWARDEE ORGANIZATION (Check All That Apply) (See GPG II.C For Definitions)
☐ SMALL BUSINESS ☐ MINORITY BUSINESS
☐ FOR-PROFIT ORGANIZATION ☐ WOMAN-OWNED BUSINESS

☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT **Floral Scents of Hybrids: Bridge or Barrier to Interspecific Gene Flow?**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ 67,551	36 months	07/01/04	

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

☐ BEGINNING INVESTIGATOR (GPG I.A)
☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C)
☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG I.B, II.C.1.d)
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☐ HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.E.1)

PI/PD DEPARTMENT

Department of Biological Sciences

PI/PD POSTAL ADDRESS

P.O. Box 2000
Graham Avenue
Cortland, NY 13045
United States

PI/PD FAX NUMBER

607-753-2927

NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Electronic Mail Address
PI/PD NAME Steven B Broyles	PhD	1992	607-753-2901	broyles@cortland.edu
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				

Electronic Signature

CERTIFICATION PAGE

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AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME **Glen C Clarke**	**Electronic Signature**	**Jan 9 2004 9:16AM**

TELEPHONE NUMBER	ELECTRONIC MAIL ADDRESS	FAX NUMBER
607-753-2511	**glenc@cortland.edu**	**607-753-5590**

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Directorate for Biological Sciences
Division of Environmental Biology
Population Biology

Proposal Classification Form
PI: Fishbein, Mark / Proposal Number: 0415895

CATEGORY I: INVESTIGATOR STATUS (Select ONE)

- ☑ Beginning Investigator - No previous Federal support as PI or Co-PI, excluding fellowships, dissertations, planning grants, etc.
- ☐ Prior Federal support only
- ☐ Current Federal support only
- ☐ Current & prior Federal support

CATEGORY II: FIELDS OF SCIENCE OTHER THAN BIOLOGY INVOLVED IN THIS RESEARCH (Select 1 to 3)

- ☐ Astronomy
- ☑ Chemistry
- ☐ Computer Science
- ☐ Earth Science
- ☐ Engineering
- ☑ Mathematics
- ☐ Physics
- ☐ Psychology
- ☐ Social Sciences
- ☐ None of the Above

CATEGORY III: SUBSTANTIVE AREA (Select 1 to 4)

- ☐ BIOGEOGRAPHY
- ☐ Island Biogeography
- ☐ Historical/ Evolutionary Biogeography
- ☐ Phylogeography
- ☐ Methods/Theory
- ☐ CHROMOSOME STUDIES
- ☐ Chromosome Evolution
- ☐ Chromosome Number
- ☐ Mutation
- ☐ Mitosis and Meiosis
- ☐ COMMUNITY ECOLOGY
- ☐ Community Analysis
- ☐ Community Structure
- ☐ Community Stability
- ☐ Succession
- ☐ Experimental Microcosms/ Mesocosms
- ☐ Disturbance
- ☐ Patch Dynamics
- ☐ Food Webs/ Trophic Structure
- ☐ Keystone Species
- ☐ COMPUTATIONAL BIOLOGY
- ☑ CONSERVATION & RESTORATION BIOLOGY
- ☐ DATABASES
- ☐ ECOSYSTEMS LEVEL
- ☐ Physical Structure
- ☐ Decomposition
- ☐ Biogeochemistry
- ☐ Limnology/Hydrology
- ☐ Climate/Microclimate
- ☐ Whole-System Analysis
- ☐ Productivity/Biomass
- ☐ System Energetics
- ☐ Landscape Dynamics
- ☐ Chemical & Biochemical Control
- ☐ Global Change
- ☐ Climate Change
- ☐ Regional Studies
- ☐ Global Studies
- ☐ Forestry
- ☐ Resource Management (Wildlife, Fisheries, Range, Other)
- ☐ Agricultural Ecology
- ☐ EXTREMOPHILES
- ☐ GENOMICS (Genome sequence, organization, function)
  - ☐ Viral
  - ☐ Microbial
  - ☐ Fungal
  - ☐ Plant
  - ☐ Animal
- ☐ MARINE MAMMALS
- ☐ MOLECULAR APPROACHES
- ☐ Molecular Evolution
- ☐ Methodology/Theory
- ☑ Isozymes/ Electrophoresis
- ☐ Nucleic Acid Analysis (general)
  - ☐ Restriction Enzymes
  - ☐ Nucleotide Sequencing
  - ☐ Nuclear DNA
  - ☐ Mitochondrial DNA
  - ☐ Chloroplast DNA
  - ☐ RNA Analysis
  - ☐ DNA Hybridization
  - ☐ Recombinant DNA
- ☐ Amino Acid Sequencing
- ☐ Gene/Genome Mapping
- ☐ Natural Products
- ☐ Serology/Immunology
- ☐ PALEONTOLOGY
- ☐ Floristic
- ☐ Faunistic
- ☐ Paleoecology
- ☐ Biostratigraphy
- ☐ Palynology
- ☐ Micropaleontology
- ☐ Paleoclimatology
- ☐ Archeozoic
- ☐ Paleozoic
- ☐ Mesozoic

- [ ] Cenozoic
- [ ] POPULATION DYNAMICS & LIFE HISTORY
- [ ] Demography/ Life History
- [ ] Population Cycles
- [ ] Distribution/Patchiness/ Marginal Populations
- [ ] Population Regulation
- [ ] Intraspecific Competition
- [ ] Reproductive Strategies
- [ ] Gender Allocation
- [ ] Metapopulations
- [ ] Extinction
- [ ] POPULATION GENETICS & BREEDING SYSTEMS
- [ ] Variation
- [ ] Microevolution
- [ ] Speciation
- [x] Hybridization
- [ ] Inbreeding/Outbreeding
- [ ] Gene Flow Measurement
- [ ] Inheritance/Heritability
- [ ] Quantitative Genetics/ QTL Analysis
- [ ] Ecological Genetics
- [ ] Gender Ratios
- [ ] Apomixis/ Parthenogenesis
- [ ] Vegetative Reproduction
- [ ] SPECIES INTERACTIONS
- [ ] Predation
- [ ] Herbivory
- [ ] Omnivory
- [ ] Interspecific Competition
- [ ] Niche Relationships/ Resource Partititioning
- [x] Pollination/ Seed Dispersal
- [ ] Parasitism
- [ ] Mutualism/ Commensalism
- [ ] Plant/Fungal/ Microbial Interactions
- [ ] Mimicry
- [ ] Animal Pathology
- [ ] Plant Pathology
- [ ] Coevolution
- [ ] Biological Control
- [ ] STATISTICS & MODELING
- [ ] Methods/ Instrumentation/ Software
- [ ] Modeling (general)
- [ ] Statistics (general)
  - [ ] Multivariate Methods
  - [ ] Spatial Statistics & Spatial Modeling
  - [ ] Sampling Design & Analysis
  - [ ] Experimental Design & Analysis
- [ ] SYSTEMATICS
- [ ] Taxonomy/Classification
- [ ] Nomenclature
- [ ] Monograph/Revision
- [ ] Phylogenetics
- [ ] Phenetics/Cladistics/ Numerical Taxonomy
- [ ] Macroevolution
- [ ] NONE OF THE ABOVE

CATEGORY IV: INFRASTRUCTURE (Select 1 to 3)

COLLECTIONS/STOCK CULTURES

- [ ] Natural History Collections
- [ ] DATABASES

FACILITIES

- [x] Controlled Environment Facilities
- [x] Field Stations
  - [ ] Field Facility Structure
  - [ ] Field Facility Equipment
- [ ] LTER Site
- [ ] INDUSTRY PARTICIPATION
- [ ] Technique Development

TRACKING SYSTEMS

- [ ] Geographic Information Systems
- [ ] Remote Sensing
- [ ] NONE OF THE ABOVE

CATEGORY V: HABITAT (Select 1 to 2)

TERRESTRIAL HABITATS

- [ ] GENERAL TERRESTRIAL
- [ ] TUNDRA
- [ ] BOREAL FOREST
- [ ] TEMPERATE
  - [x] Deciduous Forest
  - [ ] Coniferous Forest
  - [ ] Rain Forest
  - [ ] Mixed Forest
  - [ ] Prairie/Grasslands
  - [ ] Desert
- [ ] SUBTROPICAL
  - [ ] Rain Forest
  - [ ] Seasonal Forest
  - [ ] Savanna
  - [ ] Thornwoods
  - [ ] Deciduous Forest
  - [ ] Coniferous Forest
  - [ ] Desert
- [ ] TROPICAL
  - [ ] Rain Forest
  - [ ] Seasonal Forest
  - [ ] Savanna
  - [ ] Thornwoods
  - [ ] Deciduous Forest
  - [ ] Coniferous Forest
  - [ ] Desert
- [ ] CHAPPARAL/ SCLEROPHYLL/ SHRUBLANDS
- [ ] ALPINE
- [ ] MONTANE
- [ ] CLOUD FOREST
- [ ] RIPARIAN ZONES
- [ ] ISLANDS (except Barrier Islands)
- [ ] BEACHES/ DUNES/ SHORES/ BARRIER ISLANDS
- [ ] CAVES/ ROCK OUTCROPS/ CLIFFS
- [ ] CROPLANDS/ FALLOW FIELDS/ PASTURES
- [ ] URBAN/SUBURBAN
- [ ] SUBTERRANEAN/ SOIL/ SEDIMENTS
- [ ] EXTREME TERRESTRIAL ENVIRONMENT
- [ ] AERIAL

AQUATIC HABITATS

- ☐ GENERAL AQUATIC
- ☐ FRESHWATER
  - ☐ Wetlands/Bogs/Swamps
  - ☐ Lakes/Ponds
  - ☐ Rivers/Streams
  - ☐ Reservoirs
- ☐ MARINE
  - ☐ Open Ocean/Continental Shelf
  - ☐ Bathyal
  - ☐ Abyssal
  - ☐ Estuarine
  - ☐ Intertidal/Tidal/Coastal
  - ☐ Coral Reef
  - ☐ HYPERSALINE
- ☐ EXTREME AQUATIC ENVIRONMENT
- ☐ CAVES/ ROCK OUTCROPS/ CLIFFS
- ☐ MANGROVES
- ☐ SUBSURFACE WATERS/ SPRINGS
- ☐ EPHEMERAL POOLS & STREAMS
- ☐ MICROPOOLS (Pitcher Plants, Tree Holes, Other)

MAN-MADE ENVIRONMENTS

- ☐ LABORATORY
- ☐ THEORETICAL SYSTEMS
- ☐ OTHER ARTIFICIAL SYSTEMS

NOT APPLICABLE

- ☐ NOT APPLICABLE

CATEGORY VI: GEOGRAPHIC AREA OF THE RESEARCH (Select 1 to 2)

- ☐ WORLDWIDE
- ☐ NORTH AMERICA
  - ☐ United States
    - ☐ Northeast US (CT, MA, ME, NH, NJ, NY, PA, RI, VT)
    - ☐ Northcentral US (IA, IL, IN, MI, MN, ND, NE, OH, SD, WI)
    - ☐ Northwest US (ID, MT, OR, WA, WY)
    - ☑ Southeast US (DC, DE, FL, GA, MD, NC, SC, WV, VA)
    - ☐ Southcentral US (AL, AR, KS, KY, LA, MO, MS, OK, TN, TX)
    - ☐ Southwest US (AZ, CA, CO, NM, NV, UT)
    - ☐ Alaska
    - ☐ Hawaii
    - ☐ Puerto Rico
    - ☐ Canada
  - ☐ Mexico
- ☐ CENTRAL AMERICA (Mainland)
  - ☐ Caribbean Islands
  - ☐ Bermuda/Bahamas
- ☐ SOUTH AMERICA
  - ☐ Eastern South America (Guyana, Fr. Guiana, Suriname, Brazil)
  - ☐ Northern South America (Colombia, Venezuela)
  - ☐ Southern South America (Chile, Argentina, Uruguay, Paraguay)
  - ☐ Western South America (Ecuador, Peru, Bolivia)
- ☐ EUROPE
  - ☐ Eastern Europe
  - ☐ Russia
  - ☐ Scandinavia
  - ☐ Western Europe
- ☐ ASIA
  - ☐ Central Asia
  - ☐ Far East
  - ☐ Middle East
  - ☐ Siberia
  - ☐ South Asia
  - ☐ Southeast Asia
- ☐ AFRICA
  - ☐ North Africa
  - ☐ African South of the Sahara
    - ☐ East Africa
    - ☐ Madagascar
    - ☐ South Africa
    - ☐ West Africa
- ☐ AUSTRALASIA
  - ☐ Australia
  - ☐ New Zealand
  - ☐ Pacific Islands
- ☐ ANTARCTICA
- ☐ ARCTIC
- ☐ ATLANTIC OCEAN
- ☐ PACIFIC OCEAN
- ☐ INDIAN OCEAN
- ☐ OTHER REGIONS (Not defined)
- ☐ NOT APPLICABLE

CATEGORY VII: CLASSIFICATION OF ORGANISMS (Select 1 to 4)

- ☐ VIRUSES
  - ☐ Bacterial
  - ☐ Plant
  - ☐ Animal
- ☐ PROKARYOTES
  - ☐ Archaebacteria
  - ☐ Cyanobacteria
  - ☐ Eubacteria
- ☐ PROTISTA (PROTOZOA)
  - ☐ Amoebae
  - ☐ Apicomplexa
  - ☐ Ciliophora
  - ☐ Flagellates
  - ☐ Foraminifera
  - ☐ Microspora
  - ☐ Radiolaria
- ☐ FUNGI
  - ☐ Ascomycota
  - ☐ Basidiomycota
  - ☐ Chytridiomycota
  - ☐ Mitosporic Fungi
  - ☐ Oomycota
  - ☐ Zygomycota
- ☐ LICHENS
- ☐ SLIME MOLDS
- ☐ ALGAE
  - ☐ Bacillariophyta (Diatoms)
  - ☐ Charophyta
  - ☐ Chlorophyta
  - ☐ Chrysophyta
  - ☐ Dinoflagellata
  - ☐ Euglenoids
  - ☐ Phaeophyta
  - ☐ Rhodophyta
- ☐ PLANTS
  - ☐ N0N-VASCULAR PLANTS
    - ☐ BRYOPHYTA
      - ☐ Anthocerotae (Hornworts)
      - ☐ Hepaticae (Liverworts)
      - ☐ Musci (Mosses)
  - ☐ VASCULAR PLANTS
    - ☐ FERNS & FERN ALLIES
    - ☐ GYMNOSPERMS
      - ☐ Coniferales (Conifers)
      - ☐ Cycadales (Cycads)

- [ ] Ginkgoales (Ginkgo)
- [ ] Gnetales (Gnetophytes)
- [ ] ANGIOSPERMS
- [ ] Monocots
- [ ] Arecaceae (Palmae)
- [ ] Cyperaceae
- [ ] Liliaceae
- [ ] Orchidaceae
- [ ] Poaceae (Graminae)
- [x] Dicots
- [ ] Apiaceae (Umbelliferae)
- [ ] Asteraceae (Compositae)
- [ ] Brassicaceae (Cruciferae)
- [ ] Fabaceae (Leguminosae)
- [ ] Lamiaceae (Labiatae)
- [ ] Rosaceae
- [ ] Solanaceae
- [ ] ANIMALS
- [ ] INVERTEBRATES
- [ ] MESOZOA/PLACOZOA
- [ ] PORIFERA (Sponges)
- [ ] CNIDARIA
- [ ] Hydrozoa (Hydra, etc.)
- [ ] Scyphozoa (Jellyfish)
- [ ] Anthozoa (Corals, Sea Anemones)
- [ ] CTENOPHORA (Comb Jellies)
- [ ] PLATYHELMINTHES (Flatworms)
- [ ] Turbellaria (Planarians)
- [ ] Trematoda (Flukes)
- [ ] Cestoda (Tapeworms)
- [ ] Monogenea (Flukes)
- [ ] GNATHOSTOMULIDA
- [ ] NEMERTINEA (Rynchocoela) (Ribbon Worms)
- [ ] ENTOPROCTA (Bryozoa) (Plant-like Animals)
- [ ] ASCHELMINTHES
- [ ] Gastrotricha
- [ ] Kinorhyncha
- [ ] Loricifera
- [ ] Nematoda (Roundworms)
- [ ] Nematomorpha (Horsehair Worms)
- [ ] Rotifera (Rotatoria)
- [ ] ACANTHOCEPHALA (Spiny-headed Worms)
- [ ] PRIAPULOIDEA
- [ ] BRYOZOA (Ectoprocta) (Plant-like Animals)
- [ ] PHORONIDEA (Lophophorates)
- [ ] BRACHIOPODA (Lamp Shells)
- [ ] MOLLUSCA
- [ ] Monoplacophora
- [ ] Aplacophora (Solenogasters)
- [ ] Polyplacophora (Chitons)
- [ ] Scaphopoda (Tooth Shells)
- [ ] Gastropoda (Snails, Slugs, Limpets)
- [ ] Pelecypoda (Bivalvia) (Clams, Mussels, Oysters, Scallops)
- [ ] Cephalopoda (Squid, Octopus, Nautilus)
- [ ] ANNELIDA (Segmented Worms)
- [ ] Polychaeta (Parapodial Worms)
- [ ] Oligochaeta (Earthworms)
- [ ] Hirudinida (Leeches)
- [ ] POGONOPHORA (Beard Worms)
- [ ] SIPUNCULOIDEA (Peanut Worms)
- [ ] ECHIUROIDEA (Spoon Worms)
- [ ] ARTHROPODA
- [ ] Cheliceriformes
- [ ] Merostomata (Horseshoe Crabs)
- [ ] Pycnogonida (Sea Spiders)
- [ ] Scorpionida (Scorpions)
- [ ] Araneae (True Spiders)
- [ ] Pseudoscorpionida (Pseudoscorpions)
- [ ] Acarina (Free-living Mites)
- [ ] Parasitiformes (Parasitic Ticks & Mites)
- [ ] Crustacea
- [ ] Branchiopoda (Fairy Shrimp, Water Flea)
- [ ] Ostracoda (Sea Lice)
- [ ] Copepoda
- [ ] Cirripedia (Barnacles)
- [ ] Amphipoda (Skeleton Shrimp, Whale Lice, Freshwater Shrimp)
- [ ] Isopoda (Wood Lice, Pillbugs)
- [ ] Decapoda (Lobster, Crayfish, Crabs, Shrimp)
- [ ] Hexapoda (Insecta) (Insects)
- [ ] Apterygota (Springtails, Silverfish, etc.)
- [ ] Odonata (Dragonflies, Damselflies)
- [ ] Ephemeroptera (Mayflies)
- [ ] Orthoptera (Grasshoppers, Crickets)
- [ ] Dictyoptera (Cockroaches, Mantids, Phasmids)
- [ ] Isoptera (Termites)
- [ ] Plecoptera (Stoneflies)
- [ ] Phthiraptera (Mallophaga & Anoplura) (Lice)
- [ ] Hemiptera (including Heteroptera) (True Bugs)
- [ ] Homoptera (Cicadas, Scale Insects, Leafhoppers)
- [ ] Thysanoptera (Thrips)
- [ ] Neuroptera (Lacewings, Dobsonflies, Snakeflies)
- [ ] Trichoptera (Caddisflies)
- [x] Lepidoptera (Moths, Butterflies)
- [ ] Diptera (Flies, Mosquitoes)
- [ ] Siphonaptera (Fleas)
- [ ] Coleoptera (Beetles)
- [x] Hymenoptera (Ants, Bees, Wasps, Sawflies)
- [ ] Chilopoda (Centipedes)
- [ ] Diplopoda (Millipedes)
- [ ] Pauropoda
- [ ] Symphyta (Symphyla)
- [ ] PENTASTOMIDA (Linguatulida) (Tongue Worms)
- [ ] TARDIGRADA (Tardigrades, Water Bears)
- [ ] ONYCHOPHORA (Peripatus)
- [ ] CHAETOGNATHA (Arrow Worms)
- [ ] ECHINODERMATA
- [ ] Crinoidea (Sea Lilies, Feather Stars)
- [ ] Asteroidea (Starfish, Sea Stars)
- [ ] Ophiuroidea (Brittle Stars, Serpent Stars)
- [ ] Echinoidea (Sea Urchins, Sand Dollars)
- [ ] Holothuroidea (Sea Cucumbers)
- [ ] HEMICHORDATA (Acorn Worms, Pterobranchs)
- [ ] UROCHORDATA (Tunicata) (Tunicates, Sea Squirts, Salps, Ascideans)
- [ ] CEPHALOCHORDATA (Amphioxus/Lancelet)
- [ ] VERTEBRATES
- [ ] AGNATHA (Hagfish, Lamprey)
- [ ] FISHES
- [ ] Chondrichthyes (Cartilaginous Fishes) (Sharks, Rays, Ratfish)
- [ ] Osteichthyes (Bony Fishes)
- [ ] AMPHIBIA
- [ ] Anura (Frogs, Toads)
- [ ] Urodela (Salamanders, Newts)
- [ ] Gymnophiona (Apoda) (Caecilians)
- [ ] REPTILIA
- [ ] Chelonia (Turtles, Tortoises)
- [ ] Serpentes (Snakes)
- [ ] Sauria (Lizards)
- [ ] Crocodylia (Crocodilians)
- [ ] AVES (Birds)
- [ ] Passeriformes (Passerines)
- [ ] MAMMALIA
- [ ] Monotremata (Platypus, Echidna)
- [ ] Marsupalia (Marsupials)
- [ ] Eutheria (Placentals)
- [ ] Insectivora (Hedgehogs, Moles, Shrews, Tenrec, etc.)
- [ ] Chiroptera (Bats)
- [ ] Primates
- [ ] Humans
- [ ] Rodentia
- [ ] Lagomorphs (Rabbits, Hares, Pikas)
- [ ] Carnivora (Bears, Canids, Felids, Mustelids, Viverrids, Hyena, Procyonids)
- [ ] Perissodactyla (Odd-toed Ungulates) (Horses, Rhinos, Tapirs, etc.)

☐ Artiodactyla (Even-toed Ungulates) (Cattle, Sheep, Deer, Pigs, etc.) ☐ Marine Mammals (Seals, Walrus, Whales, Otters, Dolphins, Porpoises)	☐ TRANSGENIC ORGANISMS ☐ FOSSIL OR EXTINCT ORGANISMS	☐ NO ORGANISMS

CATEGORY VIII: MODEL ORGANISM (Select ONE)

☑ NO MODEL ORGANISM MODEL ORGANISM (Choose from the list)	☐ Escherichia coli ☐ Mouse-Ear Cress (Arabidopsis thaliana)	☐ Fruitfly (Drosophila melanogaster)

Directorate for Biological Sciences
Division of Environmental Biology
Population Biology

Proposal Classification Form
PI: Broyles, Steven / Proposal Number: 0415358

CATEGORY I: INVESTIGATOR STATUS (Select ONE)

- ☐ Beginning Investigator - No previous Federal support as PI or Co-PI, excluding fellowships, dissertations, planning grants, etc.
- ☑ Prior Federal support only
- ☐ Current Federal support only
- ☐ Current & prior Federal support

CATEGORY II: FIELDS OF SCIENCE OTHER THAN BIOLOGY INVOLVED IN THIS RESEARCH (Select 1 to 3)

- ☐ Astronomy
- ☐ Chemistry
- ☐ Computer Science
- ☐ Earth Science
- ☐ Engineering
- ☐ Mathematics
- ☐ Physics
- ☐ Psychology
- ☐ Social Sciences
- ☑ None of the Above

CATEGORY III: SUBSTANTIVE AREA (Select 1 to 4)

- ☐ BIOGEOGRAPHY
- ☐ Island Biogeography
- ☐ Historical/ Evolutionary Biogeography
- ☐ Phylogeography
- ☐ Methods/Theory
- ☐ CHROMOSOME STUDIES
- ☐ Chromosome Evolution
- ☐ Chromosome Number
- ☐ Mutation
- ☐ Mitosis and Meiosis
- ☐ COMMUNITY ECOLOGY
- ☐ Community Analysis
- ☐ Community Structure
- ☐ Community Stability
- ☐ Succession
- ☐ Experimental Microcosms/ Mesocosms
- ☐ Disturbance
- ☐ Patch Dynamics
- ☐ Food Webs/ Trophic Structure
- ☐ Keystone Species
- ☐ COMPUTATIONAL BIOLOGY
- ☐ CONSERVATION & RESTORATION BIOLOGY
- ☐ DATABASES
- ☐ ECOSYSTEMS LEVEL
- ☐ Physical Structure
- ☐ Decomposition
- ☐ Biogeochemistry
- ☐ Limnology/Hydrology
- ☐ Climate/Microclimate
- ☐ Whole-System Analysis
- ☐ Productivity/Biomass
- ☐ System Energetics
- ☐ Landscape Dynamics
- ☐ Chemical & Biochemical Control
- ☐ Global Change
- ☐ Climate Change
- ☐ Regional Studies
- ☐ Global Studies
- ☐ Forestry
- ☐ Resource Management (Wildlife, Fisheries, Range, Other)
- ☐ Agricultural Ecology
- ☐ EXTREMOPHILES
- ☐ GENOMICS (Genome sequence, organization, function)
  - ☐ Viral
  - ☐ Microbial
  - ☐ Fungal
  - ☐ Plant
  - ☐ Animal
- ☐ MARINE MAMMALS
- ☐ MOLECULAR APPROACHES
- ☐ Molecular Evolution
- ☐ Methodology/Theory
- ☑ Isozymes/ Electrophoresis
- ☐ Nucleic Acid Analysis (general)
  - ☐ Restriction Enzymes
  - ☐ Nucleotide Sequencing
  - ☐ Nuclear DNA
  - ☐ Mitochondrial DNA
  - ☐ Chloroplast DNA
  - ☐ RNA Analysis
  - ☐ DNA Hybridization
  - ☐ Recombinant DNA
- ☐ Amino Acid Sequencing
- ☐ Gene/Genome Mapping
- ☐ Natural Products
- ☐ Serology/Immunology
- ☐ PALEONTOLOGY
- ☐ Floristic
- ☐ Faunistic
- ☐ Paleoecology
- ☐ Biostratigraphy
- ☐ Palynology
- ☐ Micropaleontology
- ☐ Paleoclimatology
- ☐ Archeozoic
- ☐ Paleozoic
- ☐ Mesozoic

- [ ] Cenozoic
- [ ] POPULATION DYNAMICS & LIFE HISTORY
- [ ] Demography/ Life History
- [ ] Population Cycles
- [ ] Distribution/Patchiness/ Marginal Populations
- [ ] Population Regulation
- [ ] Intraspecific Competition
- [ ] Reproductive Strategies
- [ ] Gender Allocation
- [ ] Metapopulations
- [ ] Extinction
- [ ] POPULATION GENETICS & BREEDING SYSTEMS
- [ ] Variation
- [ ] Microevolution
- [ ] Speciation
- [x] Hybridization
- [ ] Inbreeding/Outbreeding
- [x] Gene Flow Measurement
- [ ] Inheritance/Heritability
- [ ] Quantitative Genetics/ QTL Analysis
- [ ] Ecological Genetics
- [ ] Gender Ratios
- [ ] Apomixis/ Parthenogenesis
- [ ] Vegetative Reproduction
- [ ] SPECIES INTERACTIONS
- [ ] Predation
- [ ] Herbivory
- [ ] Omnivory
- [ ] Interspecific Competition
- [ ] Niche Relationships/ Resource Partititioning
- [x] Pollination/ Seed Dispersal
- [ ] Parasitism
- [ ] Mutualism/ Commensalism
- [ ] Plant/Fungal/ Microbial Interactions
- [ ] Mimicry
- [ ] Animal Pathology
- [ ] Plant Pathology
- [ ] Coevolution
- [ ] Biological Control
- [ ] STATISTICS & MODELING
- [ ] Methods/ Instrumentation/ Software
- [ ] Modeling (general)
- [ ] Statistics (general)
  - [ ] Multivariate Methods
  - [ ] Spatial Statistics & Spatial Modeling
  - [ ] Sampling Design & Analysis
  - [ ] Experimental Design & Analysis
- [ ] SYSTEMATICS
- [ ] Taxonomy/Classification
- [ ] Nomenclature
- [ ] Monograph/Revision
- [ ] Phylogenetics
- [ ] Phenetics/Cladistics/ Numerical Taxonomy
- [ ] Macroevolution
- [ ] NONE OF THE ABOVE

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COLLECTIONS/STOCK CULTURES
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- [ ] LTER Site
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- [ ] Technique Development

TRACKING SYSTEMS
- [ ] Geographic Information Systems
- [ ] Remote Sensing
- [x] NONE OF THE ABOVE

CATEGORY V: HABITAT (Select 1 to 2)

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- [ ] GENERAL TERRESTRIAL
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  - ☐ Rivers/Streams
  - ☐ Reservoirs
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  - ☐ Bathyal
  - ☐ Abyssal
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  - ☐ Coral Reef
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- ☐ EPHEMERAL POOLS & STREAMS
- ☐ MICROPOOLS (Pitcher Plants, Tree Holes, Other)

MAN-MADE ENVIRONMENTS

- ☐ LABORATORY
- ☐ THEORETICAL SYSTEMS
- ☐ OTHER ARTIFICIAL SYSTEMS

NOT APPLICABLE

- ☐ NOT APPLICABLE

CATEGORY VI: GEOGRAPHIC AREA OF THE RESEARCH (Select 1 to 2)

- ☐ WORLDWIDE
- ☐ NORTH AMERICA
  - ☐ United States
    - ☐ Northeast US (CT, MA, ME, NH, NJ, NY, PA, RI, VT)
    - ☐ Northcentral US (IA, IL, IN, MI, MN, ND, NE, OH, SD, WI)
    - ☐ Northwest US (ID, MT, OR, WA, WY)
    - ☑ Southeast US (DC, DE, FL, GA, MD, NC, SC, WV, VA)
    - ☐ Southcentral US (AL, AR, KS, KY, LA, MO, MS, OK, TN, TX)
    - ☐ Southwest US (AZ, CA, CO, NM, NV, UT)
    - ☐ Alaska
    - ☐ Hawaii
    - ☐ Puerto Rico
    - ☐ Canada
  - ☐ Mexico
- ☐ CENTRAL AMERICA (Mainland)
  - ☐ Caribbean Islands
  - ☐ Bermuda/Bahamas
- ☐ SOUTH AMERICA
  - ☐ Eastern South America (Guyana, Fr. Guiana, Suriname, Brazil)
  - ☐ Northern South America (Colombia, Venezuela)
  - ☐ Southern South America (Chile, Argentina, Uruguay, Paraguay)
  - ☐ Western South America (Ecuador, Peru, Bolivia)
- ☐ EUROPE
  - ☐ Eastern Europe
  - ☐ Russia
  - ☐ Scandinavia
  - ☐ Western Europe
- ☐ ASIA
  - ☐ Central Asia
  - ☐ Far East
  - ☐ Middle East
  - ☐ Siberia
  - ☐ South Asia
  - ☐ Southeast Asia
- ☐ AFRICA
  - ☐ North Africa
  - ☐ African South of the Sahara
    - ☐ East Africa
    - ☐ Madagascar
    - ☐ South Africa
    - ☐ West Africa
- ☐ AUSTRALASIA
  - ☐ Australia
  - ☐ New Zealand
  - ☐ Pacific Islands
- ☐ ANTARCTICA
- ☐ ARCTIC
- ☐ ATLANTIC OCEAN
- ☐ PACIFIC OCEAN
- ☐ INDIAN OCEAN
- ☐ OTHER REGIONS (Not defined)
- ☐ NOT APPLICABLE

CATEGORY VII: CLASSIFICATION OF ORGANISMS (Select 1 to 4)

- ☐ VIRUSES
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  - ☐ Plant
  - ☐ Animal
- ☐ PROKARYOTES
  - ☐ Archaebacteria
  - ☐ Cyanobacteria
  - ☐ Eubacteria
- ☐ PROTISTA (PROTOZOA)
  - ☐ Amoebae
  - ☐ Apicomplexa
  - ☐ Ciliophora
  - ☐ Flagellates
  - ☐ Foraminifera
  - ☐ Microspora
  - ☐ Radiolaria
- ☐ FUNGI
  - ☐ Ascomycota
  - ☐ Basidiomycota
  - ☐ Chytridiomycota
  - ☐ Mitosporic Fungi
  - ☐ Oomycota
  - ☐ Zygomycota
- ☐ LICHENS
- ☐ SLIME MOLDS
- ☐ ALGAE
  - ☐ Bacillariophyta (Diatoms)
  - ☐ Charophyta
  - ☐ Chlorophyta
  - ☐ Chrysophyta
  - ☐ Dinoflagellata
  - ☐ Euglenoids
  - ☐ Phaeophyta
  - ☐ Rhodophyta
- ☐ PLANTS
  - ☐ N0N-VASCULAR PLANTS
    - ☐ BRYOPHYTA
      - ☐ Anthocerotae (Hornworts)
      - ☐ Hepaticae (Liverworts)
      - ☐ Musci (Mosses)
  - ☐ VASCULAR PLANTS
    - ☐ FERNS & FERN ALLIES
    - ☐ GYMNOSPERMS
      - ☐ Coniferales (Conifers)
      - ☐ Cycadales (Cycads)

      - [ ] Ginkgoales (Ginkgo)
      - [ ] Gnetales (Gnetophytes)
    - [ ] ANGIOSPERMS
      - [ ] Monocots
        - [ ] Arecaceae (Palmae)
        - [ ] Cyperaceae
        - [ ] Liliaceae
        - [ ] Orchidaceae
        - [ ] Poaceae (Graminae)
      - [x] Dicots
        - [ ] Apiaceae (Umbelliferae)
        - [ ] Asteraceae (Compositae)
        - [ ] Brassicaceae (Cruciferae)
        - [ ] Fabaceae (Leguminosae)
        - [ ] Lamiaceae (Labiatae)
        - [ ] Rosaceae
        - [ ] Solanaceae

- [ ] ANIMALS
  - [ ] INVERTEBRATES
    - [ ] MESOZOA/PLACOZOA
    - [ ] PORIFERA (Sponges)
    - [ ] CNIDARIA
      - [ ] Hydrozoa (Hydra, etc.)
      - [ ] Scyphozoa (Jellyfish)
      - [ ] Anthozoa (Corals, Sea Anemones)
    - [ ] CTENOPHORA (Comb Jellies)
    - [ ] PLATYHELMINTHES (Flatworms)
      - [ ] Turbellaria (Planarians)
      - [ ] Trematoda (Flukes)
      - [ ] Cestoda (Tapeworms)
      - [ ] Monogenea (Flukes)
    - [ ] GNATHOSTOMULIDA
    - [ ] NEMERTINEA (Rynchocoela) (Ribbon Worms)
    - [ ] ENTOPROCTA (Bryozoa) (Plant-like Animals)
    - [ ] ASCHELMINTHES
      - [ ] Gastrotricha
      - [ ] Kinorhyncha
      - [ ] Loricifera
      - [ ] Nematoda (Roundworms)
      - [ ] Nematomorpha (Horsehair Worms)
      - [ ] Rotifera (Rotatoria)
    - [ ] ACANTHOCEPHALA (Spiny-headed Worms)
    - [ ] PRIAPULOIDEA
    - [ ] BRYOZOA (Ectoprocta) (Plant-like Animals)
    - [ ] PHORONIDEA (Lophophorates)
    - [ ] BRACHIOPODA (Lamp Shells)
    - [ ] MOLLUSCA
      - [ ] Monoplacophora
      - [ ] Aplacophora (Solenogasters)
      - [ ] Polyplacophora (Chitons)
      - [ ] Scaphopoda (Tooth Shells)
      - [ ] Gastropoda (Snails, Slugs, Limpets)
      - [ ] Pelecypoda (Bivalvia) (Clams, Mussels, Oysters, Scallops)
      - [ ] Cephalopoda (Squid, Octopus, Nautilus)
    - [ ] ANNELIDA (Segmented Worms)
      - [ ] Polychaeta (Parapodial Worms)
      - [ ] Oligochaeta (Earthworms)
      - [ ] Hirudinida (Leeches)
    - [ ] POGONOPHORA (Beard Worms)
    - [ ] SIPUNCULOIDEA (Peanut Worms)
    - [ ] ECHIUROIDEA (Spoon Worms)
    - [ ] ARTHROPODA
      - [ ] Cheliceriformes
        - [ ] Merostomata (Horseshoe Crabs)
        - [ ] Pycnogonida (Sea Spiders)
        - [ ] Scorpionida (Scorpions)
        - [ ] Araneae (True Spiders)
        - [ ] Pseudoscorpionida (Pseudoscorpions)
        - [ ] Acarina (Free-living Mites)
        - [ ] Parasitiformes (Parasitic Ticks & Mites)
      - [ ] Crustacea
        - [ ] Branchiopoda (Fairy Shrimp, Water Flea)
        - [ ] Ostracoda (Sea Lice)
        - [ ] Copepoda
        - [ ] Cirripedia (Barnacles)
        - [ ] Amphipoda (Skeleton Shrimp, Whale Lice, Freshwater Shrimp)
        - [ ] Isopoda (Wood Lice, Pillbugs)
        - [ ] Decapoda (Lobster, Crayfish, Crabs, Shrimp)
      - [ ] Hexapoda (Insecta) (Insects)
        - [ ] Apterygota (Springtails, Silverfish, etc.)
        - [ ] Odonata (Dragonflies, Damselflies)
        - [ ] Ephemeroptera (Mayflies)
        - [ ] Orthoptera (Grasshoppers, Crickets)
        - [ ] Dictyoptera (Cockroaches, Mantids, Phasmids)
        - [ ] Isoptera (Termites)
        - [ ] Plecoptera (Stoneflies)
        - [ ] Phthiraptera (Mallophaga & Anoplura) (Lice)
        - [ ] Hemiptera (including Heteroptera) (True Bugs)
        - [ ] Homoptera (Cicadas, Scale Insects, Leafhoppers)
        - [ ] Thysanoptera (Thrips)
        - [ ] Neuroptera (Lacewings, Dobsonflies, Snakeflies)
        - [ ] Trichoptera (Caddisflies)
        - [ ] Lepidoptera (Moths, Butterflies)
        - [ ] Diptera (Flies, Mosquitoes)
        - [ ] Siphonaptera (Fleas)
        - [ ] Coleoptera (Beetles)
        - [ ] Hymenoptera (Ants, Bees, Wasps, Sawflies)
      - [ ] Chilopoda (Centipedes)
      - [ ] Diplopoda (Millipedes)
      - [ ] Pauropoda
      - [ ] Symphyta (Symphyla)
    - [ ] PENTASTOMIDA (Linguatulida) (Tongue Worms)
    - [ ] TARDIGRADA (Tardigrades, Water Bears)
    - [ ] ONYCHOPHORA (Peripatus)
    - [ ] CHAETOGNATHA (Arrow Worms)
    - [ ] ECHINODERMATA
      - [ ] Crinoidea (Sea Lilies, Feather Stars)
      - [ ] Asteroidea (Starfish, Sea Stars)
      - [ ] Ophiuroidea (Brittle Stars, Serpent Stars)
      - [ ] Echinoidea (Sea Urchins, Sand Dollars)
      - [ ] Holothuroidea (Sea Cucumbers)
    - [ ] HEMICHORDATA (Acorn Worms, Pterobranchs)
    - [ ] UROCHORDATA (Tunicata) (Tunicates, Sea Squirts, Salps, Ascideans)
    - [ ] CEPHALOCHORDATA (Amphioxus/Lancelet)
  - [ ] VERTEBRATES
    - [ ] AGNATHA (Hagfish, Lamprey)
    - [ ] FISHES
      - [ ] Chondrichthyes (Cartilaginous Fishes) (Sharks, Rays, Ratfish)
      - [ ] Osteichthyes (Bony Fishes)
    - [ ] AMPHIBIA
      - [ ] Anura (Frogs, Toads)
      - [ ] Urodela (Salamanders, Newts)
      - [ ] Gymnophiona (Apoda) (Caecilians)
    - [ ] REPTILIA
      - [ ] Chelonia (Turtles, Tortoises)
      - [ ] Serpentes (Snakes)
      - [ ] Sauria (Lizards)
      - [ ] Crocodylia (Crocodilians)
    - [ ] AVES (Birds)
      - [ ] Passeriformes (Passerines)
    - [ ] MAMMALIA
      - [ ] Monotremata (Platypus, Echidna)
      - [ ] Marsupalia (Marsupials)
      - [ ] Eutheria (Placentals)
        - [ ] Insectivora (Hedgehogs, Moles, Shrews, Tenrec, etc.)
        - [ ] Chiroptera (Bats)
        - [ ] Primates
          - [ ] Humans
        - [ ] Rodentia
        - [ ] Lagomorphs (Rabbits, Hares, Pikas)
        - [ ] Carnivora (Bears, Canids, Felids, Mustelids, Viverrids, Hyena, Procyonids)
          - [ ] Perissodactyla (Odd-toed Ungulates) (Horses, Rhinos, Tapirs, etc.)

☐ Artiodactyla (Even-toed Ungulates) (Cattle, Sheep, Deer, Pigs, etc.) ☐ Marine Mammals (Seals, Walrus, Whales, Otters, Dolphins, Porpoises)	☐ TRANSGENIC ORGANISMS ☐ FOSSIL OR EXTINCT ORGANISMS	☐ NO ORGANISMS

CATEGORY VIII: MODEL ORGANISM (Select ONE)

☑ NO MODEL ORGANISM MODEL ORGANISM (Choose from the list)	☐ Escherichia coli ☐ Mouse-Ear Cress (Arabidopsis thaliana)	☐ Fruitfly (Drosophila melanogaster)

Project Summary

Recent advances in the application of transgenic crops have heightened awareness of the potential ecological impacts of these emerging technologies. One area of concern is the risk of unintended gene flow from genetically modified crops to unintended target plants or wild relatives. Basic research in the evolutionary processes of speciation and hybridization may have important insights to offer investigators of this phenomenon. The behavior of insect pollinators is among the most significant factors that determine whether two populations or species are reproductively isolated. There is a rich history of research on what factors attract insects to flowers. However, only recently, with the use of powerful genetic and statistical tools, have ecologists and evolutionary biologists been able to accurately track gene flow in populations and accurately measure the contribution of individual plant traits to reproductive success. Integrative research on plant morphology, genetics, and pollinator interactions has now shown how a few plant traits, such as the color and shape of flowers, affect reproductive barriers and gene flow. However, one trait that is extremely important as a pollinator attractant, floral scent, has gone largely unstudied from this perspective.

We propose to use species of *Asclepias* (milkweeds) to investigate how floral scent is inherited by hybrids and how scents affect gene flow between species. Do the scents of hybrids accelerate or impede the rate of gene flow between species? A wealth of prior research has documented the effects of floral traits on the reproductive success of milkweeds and the roles of pollinators in mediating these effects. Milkweeds provide an illuminating system because of the rarity of successful hybridization, even though many species co-occur over vast areas. Even in the system in which we are working, in which hybridization has been well documented, there are strong barriers to hybridization. It has been hypothesized that rare F1 hybrids have morphological characteristics that bridge reproductive isolation.

We propose to rigorously test the roles of hybrids as bridges promoting gene flow between species, with emphasis on an important, but often overlooked, attribute of floral morphology—floral scent. Our experiments will provide novel insights into the phenotypic characteristics of hybrids, the underlying genetics of these traits, the effects of these traits on the patterns of mating among hybrids and their parental populations, and the impact of these mating patterns on gene flow between species. Using a combination of observational studies of natural populations, controlled crossing experiments, and controlled pollination experiments, we will integrate mechanistic and realistic explanations for the affect of hybrid scents on gene flow.

The proposed research will have significant impacts on training, outreach to underrepresented groups, and applied scientific disciplines. Mississippi State University provides an excellent opportunity to increase the research opportunities of African American students. The postdoctoral associate and undergraduate students will attend national meetings to present the results of their contributions to the project. The results of the proposed research will likely impact disciplines beyond ecology and evolutionary biology. Insights from this investigation could make a significant contribution to safely cultivating genetically engineered crops. Also, there are several threatened and endangered species of *Asclepias,* and this genus is known as a crucial food plant of the Monarch butterfly, which is of considerable conservation concern. A better understanding of the reproductive biology and hybridization dynamics of *A. exaltata* and *A. syriaca* may contribute to the conservation of rare milkweeds and the insects that depend on them.

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Appendix Items:

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

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Appendix Items:

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Project Description

Results from Prior NSF Support

The PI has not previously received NSF funding as a principal investigator.

The Co-PI (see subcontract), Dr. Steven Broyles, received the following support: NSF Grant #DEB-8914482 (1990-1992; Dissertation Improvement Grant, Dr. Robert Wyatt, PI; $7,500), “Effects of Inflorescence Size on Male and Female Reproductive Success and Pollen Dispersal in Natural and Experimental Populations of Milkweeds”. Major contributions from this research include: 1) increased understanding of the realized fitness accrued through male and female functions at the levels of whole plants and inflorescence units, by identifying seed sires using paternity-exclusion analysis, 2) quantification of long-distance pollen dispersal using paternity-exclusion analysis, 3) documentation of the loss of genetic diversity following post-glacial migration, 4) investigation of the effects of geitonogamy on female fitness, 5) investigation of the functional morphology of *Asclepias* flowers, and 6) documentation of the range expansion of *Asclepias syriaca*. The results of studies supported by this grant are reported in ten publications (Broyles and Wyatt 1990a, b, 1991, 1993, 1995, 1997; Broyles, et al. 1994; Wyatt and Broyles 1994; Wyatt, et al. 1993). Dr. Broyles also received NSF Grant #DEB-9317476 (1994-1997; RUI; $65,690) “Gene Flow and Hybridization in Milkweeds (*Asclepias* L.): A Comparison of Two Sympatric Species”. Major contributions of this research include: 1) implementation of a novel technique (allozyme and paternity-exclusion analyses of pollinia) to the identification of pollen donors in hybridizing species, 2) measuring rates of interspecific pollination, 3) implementation of multilocus genotyping to estimating the genotypic classes of F1 and backcrosses hybrids and pollinia, and 4) comparing the rates of interspecific hybridization to frequencies of F1 and backcross formation. The results of this research have been reported in three publications (Broyles, et al. 1996; Broyles 1998, 2002).

Both of these prior grants provide results that bear on the proposed research. The second grant, “Gene Flow and Hybridization in Milkweeds,” provides an important foundation for the ideas that are developed in this proposal.

Background

The importance of barriers and bridges to gene flow in the reproductive isolation between species has been underscored by concerns about genetic exchange from transgenic organisms to non-target or wild populations (Chevre, et al. 2000; Quist and Chapela 2001; Spencer and Snow 2001; Moyes, et al. 2002; Hansen et al. 2003; Messeguer 2003; Stewart, et al. 2003). These same issues are at the core of our understanding of the processes of speciation and phylogenetic reticulation. As noted by Arnold (1997), one of the focal emphases of evolutionary biologists who have investigated the significance of hybridization has been the mechanisms that limit and promote gene flow. Hybridization between species is a widespread phenomenon, and not only in plants (Grant and Grant 1992). The ecological and evolutionary significance of gene flow between species depends upon the fate of hybrid offspring. Hybrid fitness determines, in part, whether reproductive barriers between species are maintained or eroded (Grant 1971; Arnold 1997). Classical studies have explored the importance of pre- and post-zygotic barriers to interspecific gene flow. In the absence of pre-zygotic barriers, the significance of hybridization depends on the survival and fecundity of hybrids and the effects of environmental variation on hybrid fitness (Floate and Whitham 1993; Arnold and Hodges 1995; Wang, et al. 1997; Arnold, et al. 1999; Fritz 1999; Campbell, et al. 2002a; Lamont et al. 2003; Milne, et al. 2003). Pre-zygotic factors include pre- and post-pollination processes. Following interspecific pollination, the production of hybrids depends upon pollen-pistil interactions and the genetic compatibility of gametes (Carney, et al. 1994; Rieseberg, et al. 1995; Arnold 1997; Boavida, et al. 2001; Wolf, et al. 2001; Campbell, et al. 2003).

The first step that must take place in bridging reproductive barriers between species is the interspecific movement of pollen. For insect-pollinated species, pollinators must respond to floral attractants of both species and be ethologically and mechanistically capable of effecting pollination. The role of pollinators in natural plant hybridization has been widely studied (Grant 1949; Levin 1968; Arnold 1997; Campbell, et al. 1997; Leebens-Mack and Milligan 1998; Wesselingh and Arnold 2000b; Johnson 2001; Sersic, et al. 2001; Campbell, et al. 2002b; Kephart and Theiss 2003). The roles of several plant traits in promoting interspecific pollination have been investigated, including flowering phenology (Ellis and Johnson 1999; Wendt, et al. 2001; Lamont, et al. 2003), corolla morphology (Levin 1968; Campbell, et al. 1997; Campbell, et al. 1998; Meléndez-Ackerman and Campbell 1998; Ellis and Johnson 1999) and nectar characteristics (Burke, et al. 2000; Wesselingh and Arnold 2000a). However, the role of floral scents in mediating interspecific hybridization in plant populations has never been studied, despite their importance in signaling pollinators (Ayasse, et al. 2000, 2003; Thien, et al. 2000; Knudsen, et al. 2001; Kunze and Gumbert 2001; Levin, et al. 2001; Raguso 2001; Varassin, et al. 2001; Raguso and Willis 2002; Tan, et al. 2002; Andersson and Dobson 2003; Jurgens, et al. 2003; Lewis, et al. 2003; Miyake and Yafuso 2003; Raguso, et al. 2003). Floral scents have been implicated in reproductive isolation of incipient species, particularly orchids, although this has not been demonstrated under natural conditions (Dodson, et al. 1969; Gregg 1983).

The production of hybrid offspring can have a variety of impacts on the populations of parental species (Stebbins 1959; Rieseberg 1995; Arnold 1997). If backcrossing is limited (through polyploidy, chromosomal rearrangements, or novel traits that impede pollination) or disruptive selection is very strong, persistent hybrids may become new species (Ownbey and McColllum 1954; Grant 1971; Gallez and Gottlieb 1982; Rieseberg 1991, 1997; Soltis and Soltis 1993). With higher levels of backcrossing, traits may introgress into parental species, a process that has been studied extensively in diverse plant lineages (Anderson 1949; Levin 1975; Rieseberg, et al. 1988, 1995; Keim, et al. 1989; Nason, et al. 1992; Wyatt and Broyles 1992; Howard, et al. 1997; Rieseberg and Linder 1999; Morgan, et al. 2001; Rieseberg and Welch 2002; Hansen et al. 2003; Sweigart and Willis 2003; Tsukaya, et al. 2003). At the extreme, parental species identity may be eroded through introgressive hybridization (Levin, et al. 1996). Recently, attention has been focused on the mechanisms by which genetic markers and traits introgress. In particular, the floral traits of hybrids and the response of pollinators to these traits have been emphasized (Broyles, et al. 1996; Campbell, et al. 1997, 1998, 2002b; Meléndez-Ackerman and Campbell 1998; Wolf, et al. 2001). Even when hybrids have low fitness, introgression can be extensive and persistent (Arnold and Hodges 1995; Broyles, et al. 1996; Hodges, et al. 1996; Arnold, et al. 1999; Rieseberg and Linder 1999; Broyles 2002).

Common milkweed (*Asclepias syriaca*) and poke milkweed (*A. exaltata*) are broadly sympatric across eastern North America, but are found mainly in distinct habitats (Fig. 1; Woodson 1954; Broyles, et al. 1996). These species are quite distinct morphologically (Table 1) and have been classified in different infrageneric series of species (Woodson 1954), although they may be more closely related than hitherto suspected (M. Fishbein and R. Mason-Gamer, University of Illinois-Chicago, unpublished data). *A. exaltata* is found principally in forest understories, whereas *A. syriaca* is found in a variety of open habitats, including native prairies, old fields, and roadsides. Human disturbance has increased the range and probably the commonness of *A. syriaca*, providing increased opportunities for hybridization with *A. exaltata* (Wyatt, et al., 1993; Wyatt 1996). In particular, roadsides in patchily forested areas bring populations of these species into close proximity. Although *A. exaltata* typically begins to flower earlier than *A. syriaca* where they co-occur, there is usually an overlap in flowering of several weeks (Broyles, et al. 1996; Broyles 2002). Localized hybridization between these two species has been investigated at widely separated sites, using morphological (Kephart, et al. 1988), biochemical (Wyatt and Hunt 1991), and isozyme data (Wyatt and Broyles 1992; Broyles, et al. 1994, 1996; Broyles 2002). Natural hybridization between other pairs of North American species is very rare, except for that between *A. syriaca* and *A. speciosa* (Stevens 1945; Woodson 1954; Adams, et al. 1987; Wyatt and Hunt 1991; Hatfield and Kephart 2003; Kephart and Theiss 2003).

Figure 1. Geographic distribution of A. exaltata (shaded with dashed line) and A. syriaca (open with solid line) in North America. Stars represent populations where hybridization has been documented.

These previous studies of *A. exaltata* and *A. syriaca* have verified the reticulate ancestry of the presumptive hybrids, demonstrated a range of a character expression in hybrids (resemblance to one parent, intermediacy, novel traits), rate of hybrid formation, and introgression. Only a few species of Lepidoptera and honeybees have been shown to visit flowers carrying heterospecific pollen (Broyles, et al. 1996). Among the most important findings was the relationship between a rarity of hybrid formation and extensive introgression (Broyles, et al. 1996; Broyles 2002). This result indicates that backcrossing occurs at a much higher rate than F1 formation and that once hybrids form, gene flow between species can be rapid, even if the initial probability of hybridization is quite low (see also Arnold, et al. 1999). What is still unknown is the effect of hybrid morphology, including scent, on the rate and direction of interspecific gene flow.

Table 1. Distinguishing morphological characters of *A. exaltata*, *A. syriaca*, and their hybrid (from Kephart, et al. 1988). Pollen and ovule data from Wyatt, et al. (2000).

Character	*A. exaltata*	hybrid	*A. syriaca*
Lower leaf pubescence	none or sparingly pilose	moderately tomentose	densely tomentose
Leaf base	cuneate	obtuse to cuneate	rounded to obtuse
Pedicel length	long and lax	intermediate	short and rigid
Flower bud pubescence	none or with fine hairs	sparsely to moderately tomentose	densely tomentose
Flower bud apex	acute	rounded	rounded
Corolla color	greenish	green lobes, pink base	light rose to deep pink
Corolla lobe posture	strongly reflexed	reflexed with spreading tips	reflexed with spreading tips
Corona color	white	light pink	rose to pink
Corona segment shape	tubular	intermediate	hooded
Corona marginal teeth	6 (4-10)	4	2
Pollen grains/pollinium	219.1 ± 33.18	unknown	445.0 ± 30.99
Ovules/ovary	60.9 ± 10.93	unknown	208.9 ± 14.29

Project Objectives

1) Characterize the floral scents of *Asclepias exaltata*, *A. syriaca*, and their hybrids, in natural populations and in plants of known hybrid ancestry derived from controlled crosses.

2) Compare the introgression of scent characteristics to genetic markers in natural populations, by classifying plants using their multilocus genotypes.

3) Evaluate the **response of pollinators to floral scent cues** and the impacts of hybrid and parental scents on **mating patterns**, by monitoring plant reproductive success in natural populations and experimental arrays.

Project Description

This is a collaborative proposal between the PI (Fishbein, Mississippi State University) and the Co-PI (Broyles, SUNY-Cortland). The PI will oversee all aspects of project and actively participate in the field experiments, plant cultivation, and sample collection. The Co-PI will oversee sample collection and analysis of enzyme electrophoretic data and participate in field experiments, plant cultivation, and sample collection. The PI and Co-PI will be jointly responsible for data analysis the preparation of publications resulting from all aspects of the project.

Objective 1. Characterize the floral scents of *Asclepias exaltata*, *A. syriaca*, and their hybrids.

Natural populations. Parental and hybrid populations of *A. exaltata* and *A. syriaca* will be studied at Shenandoah National Park, Virginia (application for permission to work at Shenandoah has been initiated; the Co-PI has been granted permission to work in these populations in the past). This is the best studied of the localities sampled by Broyles, Wyatt, and colleagues and contains hundreds of parental plants and dozens of hybrid genets (Broyles, et al. 1994, 1996; Broyles 2002). Floral scents will be characterized for each species from ten individuals of pure parental ancestry at sites isolated from populations of the other species. These samples will serve as a baseline for assessing phenotypes that are characteristic of each species, bearing in mind that past introgression from other species cannot be excluded. Scent will also be analyzed for 100 individuals of putative pure parental ancestry, for each species, and 200 putative hybrid individuals. These presumptive hybrids will be identified using morphological characters that are diagnostic for each parental species and F1 hybrids (see Table 1; additional characters in Kephart, et al. 1988). These plants will also be used for allozyme analysis (see Objective 2). Voucher collections of parental species and hybrids will be deposited in the Mississippi State University Herbarium (MISSA).

Scents will be collected using a dynamic headspace and analyzed by combined gas chromatography-mass spectrometry (GC-MS) (Bicchi and Joulain 1990; Knudsen, et al. 1993; Dobson 1994; Raguso and Pellmyr 1998). Briefly, volatile compounds from inflorescences and vegetative structures enclosed in polyvinylacetate bags will be pulled onto an adsorbent cartridge by a vacuum. To control for artifacts introduced by the collection procedure, two different adsorbents will be employed (Raguso and Pellmyr 1998). Scent will be collected for four hours in the early afternoon, when the plants are typically pollinated. Samples of vegetative structures will serve as controls for non-floral sources of volatiles. Volatile components will be analyzed using GC-MS in the laboratory of Dr. Robert Raguso (University of South Carolina; see letter of support) and identified by comparison to mass spectral libraries.

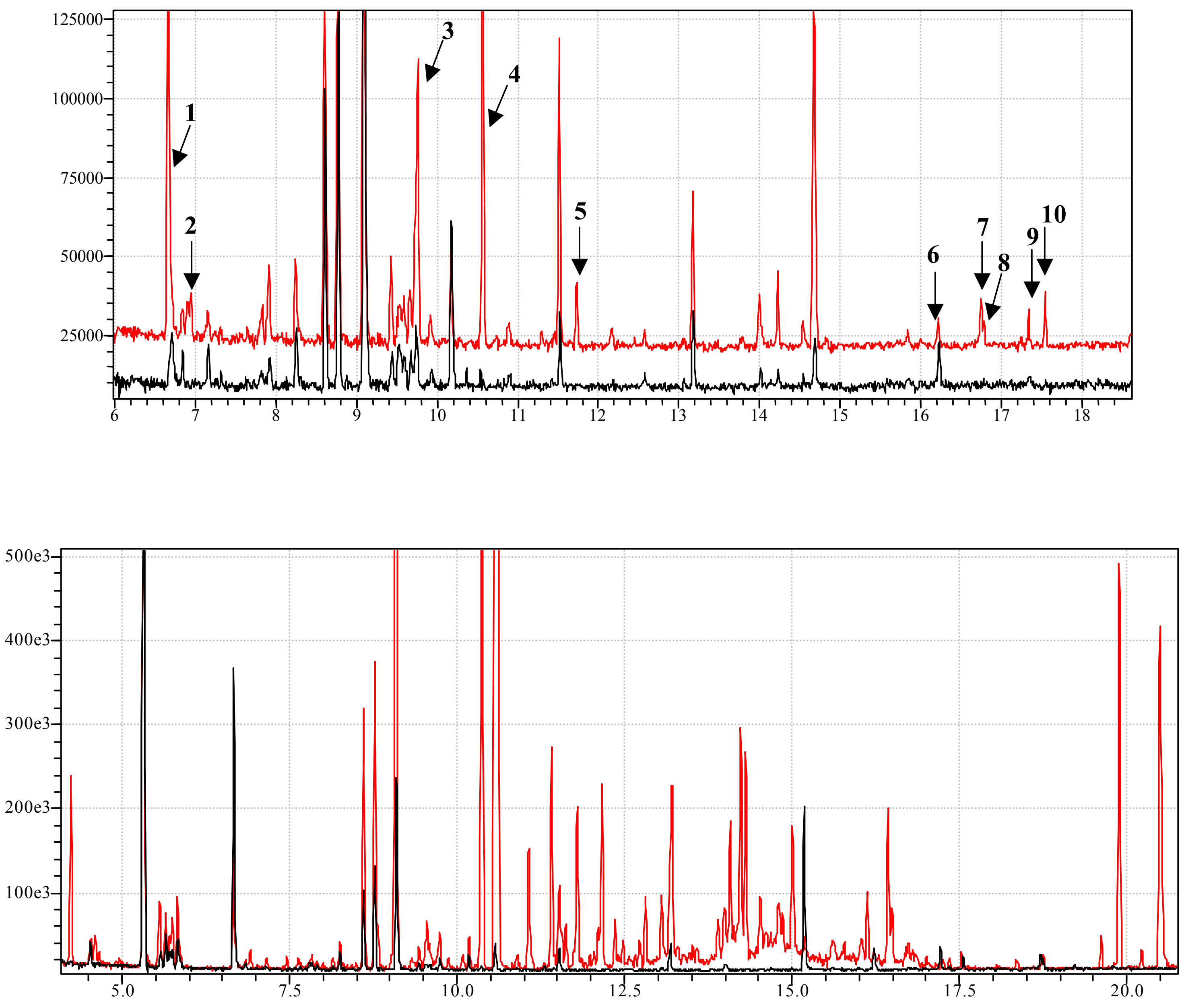
The scent characteristics of hybrids relative to parents are not well known. Classical dogma posited that hybrids should express trait values intermediate to parents (Wagner 1968). Although this outcome may be prevalent (e.g., Gregg 1983, Kuanprasert, et al. 1998; Raguso and Pichersky 1999; Raguso 2001), other possibilities are common. Many empirical examples have demonstrated that phenotypic traits of hybrids may combine parental traits, resemble one parent, be more extreme than parental trait values, or be novel relative to parents (Wilson 1992; Rieseberg and Ellstrand 1993; McDade 1995; Rieseberg 1995; Schwarzbach, et al. 2001). Commonly, hybrids are expected to exhibit combined plant chemical traits (Averett, et al. 1987). However, novel chemical traits in plant hybrids have been well documented (Ornduff, et al. 1973; Wyatt and Hunt 1991; Rieseberg and Ellstrand 1993). Secondary chemistry has been identified as a character system that may have a propensity for forming novel traits in hybrids through the alteration of biosynthetic pathways (Mears 1979; McDade 1995). The proposed research will result in a more detailed understanding of the genetics and phenotypic expression of scent characteristics in hybrids.

Experimental plants. Seeds from individuals of presumed pure parental ancestry of *A. exaltata* and *A. syriaca* (see Table 1) will be collected from mutually isolated populations, germinated, and grown to establishment (several pairs of leaves) at greenhouse facilities at Blandy Experimental Farm (University of Virginia, Boyce, VA; see attached letter of support). A sufficient number of seeds will be germinated to account for attrition and to provide leaf samples for allozyme analysis (see below) for further characterization of plants as pure parentals. At least 25 plants of each species (from at least five localities) will be transplanted outdoors at Blandy. A random sample of 10 flowering plants of each species showing pure parental allozyme genotypes will be analyzed for floral scent composition, as described above. A random sample of 5 plants of each species will be used for experimental crosses to generate F1 hybrids. Each parental individual will be crossed to each individual of the other species with each plant serving as both pollen donor and ovule donor, for a total of 50 crosses, using the techniques of Broyles, et al. (1996). Four intraspecific crosses for both species will be made to produce control plants. A sufficient number of seeds will be germinated from each cross to produce four plants that will be grown to flowering (under favorable conditions, many *Asclepias* plants, including these species, will grow from seed to flowering within 1 yr; Broyles and Wyatt 1995; M. Fishbein personal observation). Floral scent analysis will be conducted for two offspring of each of the 58 crosses (including non-hybrid controls).

Three types of second-generation crosses will be performed. F2 individuals will be produced from crosses between F1 plants. Backcrosses of F1s to each parental species will also be performed. Offspring of the intraspecific crosses described above will be used as parental plants. For all three types of crosses involving F1s, five plants will be mated in all pairwise combinations. This design will yield 25 F2s, 25 backcrosses to *A. exaltata*, and 25 backcrosses to *A. syriaca*. A sufficient number of seeds will be germinated from each cross to produce four plants that will be grown to flowering and floral scent analysis will be conducted for two offspring of each of the 75 crosses. These analyses will give us the most stringent possible controls for the comparison of scent composition of parental species and hybrids and to evaluate scent variation across environments.

Preliminary analyses show striking differences in the scent compositions of these species (Fig. 2). Floral volatiles of both parental species were collected and analyzed by Ms. Tracey Slotta (Virginia Polytechnic Institute and State University) and Dr. Raguso. We consider Ms. Slotta an ideal candidate for the postdoctoral research associate position to be funded by this project (see Budget Justification). She is familiar with the parental species, adept at scent collection protocols, and will receive her Ph.D. in May 2004. The scent analysis of *A. syriaca* revealed a remarkable diversity of volatile compounds, many of which are present in large quantities. There are several compounds that are common pollinator attractants. In particular, benzaldehyde and 2-phenyl-ethanol are present in very large quantities, are easily detectable on chromatograms, and will be easy to track if they introgress into *A. exaltata*, which lacks these compounds. It is also noteworthy that there is a large number of compounds (>50), some of which have not yet been identified (by comparison to a library of 120,000 mass spectra) and have not previously been detected in floral scents (R. Raguso, personal communication). The scent of *A. exaltata* is quite different. Only a few compounds are present and only in barely detectable

Figure 2. GC traces of *Asclepias exaltata* (upper panel) and *Asclepias syriaca* (lower panel) floral scent compounds (upper, red) and volatiles collected from vegetative structures (lower, black). Note the difference in vertical scale of the two chromatograms. Significant floral peaks not present in vegetative material are numbered for *A. exaltata*, but not *A. syriaca*, because of the large number of peaks in the latter species. Data collected and analyzed by T. Slotta and R. Raguso.



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concentrations (e .g. ocimene, sesquiterpenes). It is possible that stronger and more complex scents were not detected in *A. exaltata* because samples were collected at the very end of the flowering season during a period of sporadic precipitation. We will increase sample collection time to increase the concentration of components that may have fallen beneath the detection threshold in our preliminary analysis.

Objective 2. Compare the introgression of scent characteristics to genetic markers.

One hundred individuals of *A. exaltata* and *A. syriaca* of pure parentage sampled for scent analysis, as described in Objective 1, will be genotyped. The multilocus genotype of each of these 200 plants will be assayed using standard allozyme techniques as described by the Co-PI (Broyles and Wyatt 1990a, 1993, 1995; Broyles, et al. 1994, 1996; Broyles 2002). In addition to the pure parental plants, a sample of 200 putatively hybrid individuals analyzed for scent composition, as described in Objective 1, and will be assayed for their multilocus genotype. Populations of *A. exaltata* and *A. syriaca* possess high levels of allozyme variation. Furthermore, the two species differ in several alleles and allele frequencies (Table 2). For example, alternative *Mdh* alleles (*Mdh-2b* for *A. exaltata* and *Mdh-2a* for *A. syriaca*) are found in high frequencies in one species, but are essentially non-existent in the second. Thirty-one informative allozymes are quickly and easily scored for 10 loci using two buffer systems. These species-informative alleles permit hybrid genotypes to be identified and assigned to one of six genotypic classes (parentals, F1s, F2s, and first generation backcrosses to each parent) using the maximum likelihood method of Nason and Ellstrand (1993). This level of polymorphism and interspecific variation allows genotypic classes to be assigned with a high degree of confidence for these milkweeds and their hybrids (Broyles 2002). All genotype assays will be conducted in the Co-Pi's laboratory at SUNY-Cortland.

The distributions of scent compounds and allozymes will be compared in two ways. First, the frequencies of apparently introgressed scent compounds (i.e., those diagnostic of the other species in isolated populations) will be compared to the frequencies of apparently introgressed alleles in the parental samples. This comparison will evaluate the similarity in direction and strength of the introgression of allozymic and scent characters. By making the assumption, ceteris paribus, that allozymic variation is often neutral, deviations between allozymic and scent introgression will be indicative of selection on scent characteristics. Second, the distribution of scent characteristics in the six genotypic classes (2 parentals, F1s, F2s, 2 F1-parental backcrosses) will give some indication of the genetic and phenotypic properties of scent. For example, we will ask whether different classes of hybrids are intermediate, transgressive, are similar to parentals in scent characteristics. These data will complement our assessment of scent inheritance in Objective 1 by examining plants exhibiting a potentially broader range of natural variation.

Table 2. Frequencies of species informative alleles (**bold**) from allopatric populations of *Asclepias exaltata* and *A. syriaca* in northern Shenandoah National Park, VA. Allele frequencies are based on more than 400 genotypes for each species. Data reported, in part, in Broyles (2002).

Locus	Alleles	*A. exaltata*	*A. syriaca*
Idh-1	a	**0.110**	---
	c	0.034	**0.500**
Mdh-1	a+	---	**0.005**
	a	0.012	**0.995**
	b	**0.998**	---
Mdh-2	a	0.090	**1.000**
	b	**0.910**	
Mnr-1	a	**0.256**	0.019
	c	**0.435**	0.001
	e	**0.005**	----
Mnr-2	a	**0.069**	----
	c	**0.003**	----
Pgd-1	a++	----	**0.055**
	a+	----	**0.015**
	a	**0.115**	----
	c	**0.329**	0.010
	d	**0.001**	----
	e	**0.006**	----
Pgi-2	a	**0.095**	----
	c	**0.006**	----
	d	**0.077**	----
Pgm-1	a	**0.110**	0.005
	c	**0.179**	0.015
	d	0.012	**0.385**
	e	**0.003**	----
	f	**0.023**	----
Tpi-1	a	**0.007**	----
	c	**0.015***	----
	d	**0.005***	----
Tpi-2	a	**0.012***	----
	c	0.001	**0.093**

Objective 3. Evaluate the response of pollinators to floral scent cues and the impacts of hybrid and parental scents on mating patterns

Natural populations. Ten parental plants of each species and 20 hybrid plants sampled for scent characteristics (Objective 1) will be marked and used the following year as focal plants for measuring rates of pollinator visitation and interspecific pollinator movement. Note that these plants will have been genotyped and assigned to parental, hybrid, and backcross classes as well. Inflorescences will be bagged prior to observation. Focal plants will be unbagged and observed for 30 min periods, during which time all flower visitors will be recorded, identified, and the duration of the visit timed using methods described in Fishbein and Venable (1996a). Pollinators will be identified to the least inclusive taxon as is practical from a distance (see e.g., Fishbein and Venable 1996a). Voucher specimens will be deposited in the Mississippi Entomological Museum, Mississippi State University. The identity of the next plant visited will also be noted (*A. syriaca, A. exaltata,* hybrid). Thirty individuals each of the remaining parental plants of each species and hybrids will be monitored for the number and genotype (as in Objective 2) of pollinia inserted, using the techniques of Broyles and Fishbein (Broyles and Wyatt 1990a, b; 1995; Broyles, et al. 1996; Fishbein and Venable 1996a, b; Broyles 2002). Flowers will be harvested and stored at 4C for subsequent pollinia removal and enzyme extraction (Shore 1993; Broyles 2002). Pollen donors will be identified by the multilocus genotype of pollinia (because pollinia contain hundreds of replications of the meiotic process, pollinia genotypes represent the diploid genotype of the pollen donors). The high levels of allozyme polymorphism (Table 2) provide an excellent opportunity for unambiguous identification of pollen donors. The remaining 60 plants of each parental species and 150 hybrid plants will be assayed for fruit production. Seeds from mature fruits will be genotyped and their pollen parent identified using paternity exclusion analysis (Broyles and Wyatt 1990a, b, 1995). Because fruits with multiple paternities are rare, siring success is accurately measured from a subsample of seeds from a fruit (Broyles and Wyatt 1990a, b; Broyles, et al. 1994; Gold and Shore 1995). As noted, the high levels of variability provide robust analyses of paternity in these milkweeds. These data will be used to test for differences among genotypes and scent phenotypes in components of reproductive success through male and female function (pollinator visitation, pollen donation, pollen receipt and seed production). The data will also permit evaluation of the relationships between patterns of pollinator visitation and interspecific gene flower. Most importantly, we will be able to evaluate how the scent characteristics of hybrids and parentals influence the patterns of reproductive success and gene flow.

Experimental plants: *Randomized arrays*. Using plants grown from seed and genotyped in the same manner as described in Objective 1, hexagonal arrays of 36 pots (12 each of flowering *A. syriaca*, *A. exaltata*, and hybrid; see Broyles and Wyatt 1995) will be placed in the field at Shenandoah N.P. Hybrids will be identified by morphology (Table 1) and genotype. Only hybrids classified as F1s by multilocus genotype will be used. Three of these replicate arrays will be created. Pots of *A. exaltata* and hybrids will be paired and umbels from paired plants tied together to make inflorescences comparable in flower number to those of *A. syriaca* (see Fishbein and Venable 1996b). In addition "extra" umbels on *A. syriaca* will be enclosed in polyvinylacetate bags only during the period of pollinator observation to contain scent and restrict access to nectar. Arrays will be placed in the vicinity of dense populations of parental and hybrid plants to aid in drawing pollinators to the experimental plants.

A commercially available (Koppert Biological Systems) pollinator, *Bombus impatiens*, will be employed in preference studies of parental and hybrid plants (Kearns and Inouye 1993). *B. impatiens* is native to the eastern U. S., which minimizes concern over its release in a field experiment. It has been noted as a common visitor to both *A. syriaca* and *A. exaltata*, as well as other species of *Asclepias* (Kephart 1983; Broyles and Wyatt 1995). Experimental inflorescences (which have been matched for flower number) will be bagged with bridal veil prior to observation to minimize nectar removal and pollen transfer (Wyatt, et al. 1992; Fishbein and Venable 1996a, b). These bags will be removed at the commencement of observation. At this time, teams of observers will monitor a subset of experimental plants, recording visitation data (taxon, duration, etc.) for three 30 min periods staggered through the first day of observation. Observations will be repeated daily for 7 days (approximate floral longevity of these species). Flowers on experimental plants will be harvested and pollinia removed, as described above for the natural population. Pollinia will be genotyped and assigned to parental or hybrid classes. This experiment will complement the natural experiment by controlling for plant density, neighborhood, display size, and variation among pollinator species. The large number of released *Bombus impatiens* will swamp out the effects of naturally occurring pollinators at our experimental patch. As with the natural experiment, these data will be used to test for differences among genotypes and scent phenotypes in mating patterns, and to assess the role of hybrids in this process.

Experimental plants: *Hybrids in uniform backgrounds*. There is tremendous variation in the population context in which hybrids occur. At Shenandoah N.P., most hybrids are embedded in populations of *A. syriaca* (Broyles, et al. 1996; Broyles 2002). The evolutionary potential for introgression between *A. syriaca* and *A. exaltata* depends heavily on the reproductive success of F1 hybrids in the milieu of *A. syriaca*. This pattern of hybrid establishment appears to be an artifact of *A. syriaca* serving as the maternal parent in interspecific crosses rather than a product of habitat selection operating on hybrids (Broyles 2002). However, in other areas, this bias does not occur, and hybrids may find themselves in closer proximity to *A. exaltata*. Shenandoah populations show a preponderance of introgression from *A. exaltata* to *A. syriaca* (Broyles 2002), but other populations show evidence of introgression in the opposite direction (Kephart, et al. 1988; Wyatt and Broyles 1992; Broyles and Wyatt 1993). Previous investigations have not unraveled how the relative density of parental species affects mating patterns and introgression. In the present context, we seek to discover the role that floral traits (especially scents) play in this dynamic. Is the effect of hybrid morphology on mating patterns and introgression dependent on the context of background parental populations?

Forty hybrid plants grown from seed collected from a hybrid parent, as described for the array experiment, will be selected by screening for F1-classed genotypes. Each plant will be assayed for floral scent composition. Hybrids will be rotated through three naturally occurring population structures (pure *A. syriaca*, pure *A. exaltata*, hybrid swarm) for two-day intervals. Pollinator visitation and interplant pollinator flights will be recorded for hybrid plants and compared with a randomly selected control plant from the natural population. Open flowers will be harvested and pollinia removed from stigmatic chambers at the end of the second day for subsequent allozyme analysis and genotypic classification. From this study, we will determine the interacting effects of hybrid scent and background population composition on mating patterns and direction and strength of introgression.

Arrays of artificial flowers. Artificial flowers will be constructed by drilling wells into Plexiglas and painting simple pentagonal shapes (Harder 1988; Kearns and Inouye 1993; Church, et al. 2001; Keasar, et al. 2002; Wiegmann, et al. 2003). White and pink paint will be used to simulate the coloration of *A. exaltata* and *A. syriaca* flowers. Flowering plants, isolated from one another, will be positioned beneath the wells to provide scent cues. Arrays of six "flowers" will be used. Initially, arrays will be stocked with all one species' scent. Following release of commercial *B. impatiens*, which has been shown to learn quickly to visit artificial flowers (Church, et al. 2001), the first array will be exposed to pollination and visitation rates will be measured over a 5 min period, following a 10 min acclimation period. The array will be replaced with another stocked with the second species scent. After a 10 min refractory period, visitation rate will be measured for 5 min. Arrays will be rotated for a total of 30 pairs of observations. The experiment will be replicated using randomly mixed arrays of three plants of each species, for 30 more observations. For mixed array experiments, between "species" movements will be recorded in addition to visitation rates to each kind of artificial flower. These experiments will be repeated with colors and odors swapped, to test for color effects and interactions between scent and color. The entire battery of experiments will be replicated in a flight cage, with a single, randomly caught bumblebee. Bees will be released after each trial and a new bee caught at random. These experiments will test for pollinator scent preferences under conditions that control for all other plant characteristics. These data will give more mechanistic insights into how scent affects pollinator movements and gene flow.

Significance of the Proposed Research

A wealth of prior research has documented the effects of floral morphology and display on the reproductive biology of milkweeds and the roles of pollinators in mediating these effects. Studies have begun to be extended to a process-level understanding of how reproductive barriers break down between species and how introgression occurs. The mechanisms and dynamics of hybridization and introgression are not well understood for many kinds of plants. Milkweeds provide an illuminating system for these studies because of the extreme rarity of successful hybridization in the vast areas of North America in which multiple species are sympatric. Clearly, reproductive isolation between species is quite strong. Even in the system in which we are working, in which hybridization has been well documented, there are strong barriers to hybridization. Paradoxically, advanced generations of hybridization appear to occur rapidly, despite the barriers to initial hybrid formation. It has been hypothesized that rare F1 hybrids have morphological characteristics that bridge reproductive isolation, accelerating the rate of hybridization, and consequently rates of introgression.

We propose to rigorously test the roles of hybrids as bridges promoting gene flow between species, with emphasis on an important, but often overlooked attribute of floral morphology—floral scent. Our experiments will provide novel insights into the phenotypic characteristics of hybrids, the underlying genetics of these traits, the effects of these traits on the patterns of mating among hybrids and their parental populations, and the impact of these mating patterns on gene flow between species. Using a combination of observational studies of natural populations, controlled crossing experiments, and controlled pollination experiments, we will endeavor to achieve an integration of mechanistic and realistic explanations for the significance of hybrid scents to interspecific gene flow.

Broader impacts of proposed research

The proposed research will have significant impacts on **training**, **outreach to underrepresented groups**, and **applied scientific disciplines**. The project will train the postdoctoral associate in floral scent analysis, allozyme analysis, and pollination ecology, the PI in floral analysis, and undergraduate students at two universities in a variety of approaches to field and laboratory biology. The project will target underrepresented groups. Mississippi State University provides an excellent opportunity to increase the research opportunities of African American students, as underscored by current NSF funding for an REU site in conservation biology in which the PI is participating. Because a portion of study will be undertaken Blandy Experimental Farm, which is part of the State Arboretum of Virginia, there is the potential for public education during the course of the study. The postdoctoral associate will also gain experience in mentoring undergraduates. The postdoc and students will attend national meetings to present the results of their contributions to the project. The results of the proposed research will likely impact disciplines beyond ecology and evolutionary biology. Insights gained from this investigation of the roles of floral scents and pollinators in gene flow between species could make a significant contribution to safely cultivating genetically engineered crops. Also, there are several threatened and endangered species of *Asclepias,* and this genus is known as a crucial food plant of the Monarch butterfly, which is of considerable conservation concern. A better understanding of the reproductive biology and hybridization dynamics of *A. exaltata* and *A. syriaca* may contribute to the conservation of rare milkweeds and the insects that depend on them.

Research Plan

Yr 1	June-July 2004	1) Collect scent from isolated parental populations 2) Collect scent and leaf samples from parental and hybrid plants in sympatry at Shenandoah N.P. 3) Assay allozymes from leaf samples from Shenandoah
	Aug. 2004	1) Analyze scent samples
	Sept.-Oct. 2004	1) Collect seed from isolated parental populations for experimental crosses 2) Germinate seeds and begin growing in pots at Blandy Experimental Farm greenhouse
	May 2005	1) Transfer nursery plants outdoors
Yr 2	June-July 2005	1) Collect scent from flowering experimental plants at Blandy 2) Conduct cross pollinations at Blandy to produce first generation 3) Transfer nursery plants for pollination studies to Shenandoah NP 4) Conduct pollinator visitation observations 5) Collect flowers for inserted pollinia samples 6) Assay allozymes from pollinia samples
	Aug. 2005	1) Analyze scent samples from Blandy
	Sept.-Oct. 2005	1) Assay fruit production and collect seeds for allozyme analysis from pollination study at Shenandoah 2) Collect seed from cross pollinations at Blandy 3) Germinate seeds and begin to grow in pots at Blandy
	May 2006	1) Transfer nursery plants outdoors
Yr 3	June-July 2006	1) Collect scent from flowering first generation crosses at Blandy 2) Conduct cross pollinations at Blandy to produce second generation 3) Continue pollination studies at Shenandoah, if necessary
	Aug. 2006	1) Analyze scent samples from Blandy
	Sept.-Oct. 2006	1) Collect seed from cross pollinations at Blandy 2) Germinate seeds and begin to grow in pots at Blandy
	May 2007	1) Transfer nursery plants outdoors
	June-July 2007	1) Collect scent from flowering second generation crosses at Blandy 2) Analyze scent samples from Blandy

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Wesselingh, R. A., and M. L. Arnold. 2000a. Nectar production in Louisiana iris hybrids. International Journal of Plant Sciences 161:245-251.
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Wolf, P. G., D. R. Campbell, N. M. Waser, S. D. Sipes, T. R. Toler, and J. K. Archibald. 2001. Tests of pre- and postpollination barriers to hybridization between sympatric species of *Ipomopsis* (Polemoniaceae). American Journal of Botany 88:213-219.
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Wyatt, R., and S. B. Broyles. 1992. Hybridization in North American *Asclepias*. III. Isozyme evidence. Systematic Botany 17:640-648.
Wyatt, R., and S. B. Broyles. 1994. Ecology and evolution of reproduction in milkweeds. Annual Review of Ecology and Systematics 25:423-441.
Wyatt, R., S. B. Broyles, and G. S. Derda. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). American Journal of Botany 79:636-642.
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Wyatt, R. and D. M. Hunt. 1991. Hybridization in North American *Asclepias*. II. Flavonoid evidence. Systematic Botany 16:132-142.
Wyatt, R., A. Stoneburner, S. B., Broyles, and J. R. Allison. 1993. Range extension southward in common milkweed, *Asclepias syriaca* L. Bulletin of the Torrey Botanical Club 120:177-179.

Biographical Sketch: Mark Fishbein

Professional Preparation

University of Illinois-Chicago	Biological Sciences	B.S.	1987
University of Arizona	Ecology and Evolutionary Biology	M.S.	1991
University of Arizona	Ecology and Evolutionary Biology	Ph.D.	1996
Washington State University	Plant Systematics and Evolution	1998-2000	
University of Idaho	Plant Systematics	2000-2001	

Appointments

Mississippi State University, Assistant Professor and Herbarium Director Department of Biological Sciences	2001-present
University of Idaho, Postdoctoral Instructor and Researcher Department of Biological Sciences	2000-2001
Washington State University, Visiting Assistant Professor Departments of Botany and Zoology	1997-1998
Earlham College, Instructor	1997
University of Arizona, Adjunct Assistant Professor Department of Ecology and Evolutionary Biology	1996

Publications
Most closely related to project

1) Fishbein, M. 2001. Evolutionary innovation and diversification in the flowers of Asclepiadaceae. *Annals of the Missouri Botanical Garden* 88:603-623.
2) Fishbein, M. and S.P. Lynch. 1999. *Asclepias jorgeana* (Asclepiadaceae), a new milkweed from montane western México. *Novon* 9:179-184.
3) Fishbein, M. 1997. A new name in Mexican *Asclepias* (Asclepiadaceae). *Novon* 7:234.
4) Fishbein, M. and D.L. Venable. 1996. Diversity and temporal change in the effective flower visitors of *Asclepias tuberosa*. *Ecology* 77:1061-1073.
5) Fishbein, M. and D.L. Venable. 1996. Evolution of inflorescence design: theory and data. *Evolution* 50:2165-2177.

Other significant publications

1) Fishbein, M. and D. E. Soltis. Further resolution of the rapid radiation of Saxifragales(angiosperms, eudicots) supported by Bayesian analysis. *Systematic Botany*. In press.
2) Soltis, D. E., M. Fishbein, and R. K. Kuzoff. 2003. Reevaluating the evolution of epigyny: data from phylogenetics and floral ontogeny. *International Journal of Plant Sciences* 164 (Suppl. 5):S251-S264
3) Fishbein, M., C. Hibsch-Jetter, D. E. Soltis, and L. Hufford. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): analysis of a rapid, ancient radiation. *Systematic Biology* 50:817-847.
4) Soltis, D. E., R. K. Kuzoff, M. E. Mort, M. Zanis, M. Fishbein, L. Hufford, J. Koontz, and M. K. Arroyo. 2001. Elucidating deep-level relationships in Saxifragaceae using sequences for six chloroplastic and nuclear DNA regions. *Annals of the Missouri Botanical Garden* 88:669-693.
5) Fishbein, M. and R. Levin. 1997. *Metastelma mexicanum* (Asclepiadaceae): a new combination and re-evaluation of the status of *Basistelma* Bartlett. *Madroño* 44:268-276.

Synergistic Activities

1) Initiation of a computerized database of specimens in the Mississippi State University Herbarium
2) Mentoring and employment of an African American undergraduate woman as a laboratory researcher in molecular systematics
3) Participant in an NSF REU site to provide research experience to underrepresented groups at small colleges in Mississippi and neighboring states
4) Establishment of a departmental instructional stereomicroscope facility

Collaborators and Other Affiliations

Collaborators in last 48 months: **Dr. Anurag Agrawal**, University of Toronto, **Dr. Mary Kalin Arroyo**, Universidad de Chile, **Dr. Steven B. Broyles**, State University of New York-Cortland, **Ms. Carola Hibsch-Jetter**, Würzburg, Germany, **Dr. Larry Hufford**, Washington State University, **Dr. Hasan Jamil**, Mississippi State University, **Dr. Jason Koontz**, Illinois Natural History Survey, **Dr. Robert Kuzoff**, University of Georgia, **Dr. Stephen P. Lynch**, Louisiana State University-Shreveport, **Dr. Roberta Mason-Gamer**, University of Illinois-Chicago, **Dr. Mark E. Mort**, Kansas University, **Dr. Robert Raguso**, University of South Carolina, **Dr. Douglas E. Soltis**, University of Florida, **Mr. Michael Zanis**, Washington State University

Graduate and postdoctoral advisors: Dr. D. Lawrence Venable (Graduate), University of Arizona, University of Illinois-Chicago, **Dr. Douglas E. Soltis**, (Postdoctoral), University of Florida, **Dr. Larry Hufford** (postdoctoral), Washington State University, **Dr. Roberta Mason-Gamer** (postdoctoral), University of Idaho

Thesis advisor and postgraduate sponsor: Mr. Chris Doffitt (Ph.D.), **Ms. Margaret Parks** (M.S.)

STEVEN BRIAN BROYLES
Associate Professor of Biological Sciences
Department of Biological Sciences
State University of New York College at Cortland
Cortland, NY 13045

Professional Preparation

University of North Carolina at Charlotte	Biology	B. S. 1983
University of Georgia	Botany	M.S. 1988
University of Georgia	Botany	Ph. D. 1992

Appointments

1998-present Associate Professor, SUNY Cortland
1992-1997 Assistant Professor, SUNY Cortland

Representative Publications

Broyles, S. B. 2002. Hybrids as bridges to gene flow in milkweeds. *Evolution* 56: 1943-1953.

Wyatt, R., **S. B. Broyles**, and S. R. Lipow. 2000. Pollen-ovule ratios in milkweeds. *Bulletin of the Torrey Botanical Club* 25:171-180.

Broyles, S.B. 1998. Post-glacial migration and the loss of allozyme variation in northern populations of *Asclepias exaltata. American Journal of Botany* 85: 1091-1097.

Broyles, S. B., S. L. Sherman-Broyles, and C. Vail. 1996. Pollination genetics of hybridization in sympatric populations of *Asclepias exaltata* and *Asclepias syriaca* (Asclepiadaceae). *American Journal of Botany* 83:1580-1584.

Broyles, S. B. and R. Wyatt. 1995. A reexamination of the "pollen donation hypothesis" in an experimental population of *Asclepias exaltata. Evolution* 49:89-99.

Broyles, S. B., A. Schnabel, and R. Wyatt. 1994. Evidence for long-distance pollen dispersal and interspecific pollen transfer in milkweeds (*Asclepias exaltata*). *Evolution* 48:1032-1040.

Wyatt, R. and **S. B. Broyles.** 1994. The ecology and evolution of reproduction in milkweeds (*Asclepias*). *Annual Review of Ecology and Systematics* 25:423-441.

Broyles, S. B. and R. Wyatt. 1993. Allozyme diversity and genetic population structure in Poke Milkweed, *Asclepias exaltata. Systematic Botany* 18:18-30.

Wyatt, R. and **S. B. Broyles.** 1992. Hybridization in North American *Asclepias*. III. Isozyme evidence. *Systematic Botany* 17:640-648.

Broyles, S. B. and R. Wyatt. 1991. Effective pollen dispersal in a natural population of *Asclepias exaltata:* The influence of pollinator behavior, genetic similarity, and mating success. *American Naturalist* 138:1239-1249.

Synergistic Activities

1996-1998	Restructuring of Science Education for Elementary Education Majors at SUNY Cortland. This project was sponsored by NSF. My contributions included writing curricular materials for a new integrated Earth Science/Life Science course.
2000-2001	SUNY Cortland Adolescents Education Accreditation. I have served as the Departmental representative for assembling Program Reviews for National Accreditation. This also includes writing educational rubrics and collecting data for the review process.
1999-2001	Board Member and Contributor to Newsletter Publications of Lime Hollow Nature Center in Cortland County, New York.
2002-Present	Board Member and current Chairperson of the City of Cortland Advisor Waterboard. Current member of City of Cortland Landscape Design Commisssion.

Collaborators and Other Affliations

Collaborators	Mr. John Baran	graduate student at Cornell University
	Mrs. Susan Sherman	graduate student at Cornell University
	Dr. Peter Ducey	SUNY Cortland
	Dr. Lawrence Klotz	SUNY Cortland
	Dr. Sara Lipow	unknown
	Dr. Robert Wyatt	Highlands Biological Station, NC
	Ms. Michelle Dean	student SUNY Cortland
Graduate Advisor	Dr. Robert Wyatt	Highlands Biological Station

SUMMARY PROPOSAL BUDGET   YEAR 1

ORGANIZATION **Mississippi State University**				FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Mark Fishbein**				AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Mark Fishbein - Assistant Professor	0.00	0.00	1.00	$ 4,802	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	4,802	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (1) POST DOCTORAL ASSOCIATES	12.00	0.00	0.00	32,000	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (2) UNDERGRADUATE STUDENTS				4,200	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				41,002	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				9,427	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				50,429	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) Desktop Computer $ 6,000					
TOTAL EQUIPMENT				6,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				9,168	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				12,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				17,157	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				29,157	
H. TOTAL DIRECT COSTS (A THROUGH G)				94,754	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Direct Costs (Rate: 43.0000, Base: 88754)					
TOTAL INDIRECT COSTS (F&A)				38,164	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				132,918	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 132,918	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME **Mark Fishbein**	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* **Lynda Tuck**	Date Checked	Date Of Rate Sheet	Initials - ORG

1 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

0415895

SUMMARY PROPOSAL BUDGET

YEAR 2

ORGANIZATION **Mississippi State University**				FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Mark Fishbein**				AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Mark Fishbein - Assistant Professor	0.00	0.00	1.00	$ 4,994	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	4,994	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (1) POST DOCTORAL ASSOCIATES	12.00	0.00	0.00	33,280	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (2) UNDERGRADUATE STUDENTS				4,368	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				42,642	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				9,879	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				52,521	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				11,704	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				7,700	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				1,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				24,859	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				33,559	
H. TOTAL DIRECT COSTS (A THROUGH G)				97,784	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) Direct Costs (Rate: 43.0000, Base: 72925)					
TOTAL INDIRECT COSTS (F&A)				31,358	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				129,142	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 129,142	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME **Mark Fishbein**	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* **Lynda Tuck**	Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

0415895

SUMMARY PROPOSAL BUDGET — YEAR 3

ORGANIZATION **Mississippi State University**				FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Mark Fishbein**				AWARD NO.		
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)	
1. **Mark Fishbein - Assistant Professor**	0.00	0.00	1.00	$ 5,194	$	
2.						
3.						
4.						
5.						
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0		
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	5,194		
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)						
1. (1) POST DOCTORAL ASSOCIATES	12.00	0.00	0.00	34,611		
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0		
3. (0) GRADUATE STUDENTS				0		
4. (2) UNDERGRADUATE STUDENTS				4,543		
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0		
6. (0) OTHER				0		
TOTAL SALARIES AND WAGES (A + B)				44,348		
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				10,194		
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				54,542		
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)						
TOTAL EQUIPMENT				0		
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				14,913		
2. FOREIGN				0		
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0						
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0		
G. OTHER DIRECT COSTS						
1. MATERIALS AND SUPPLIES				6,000		
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				1,000		
3. CONSULTANT SERVICES				0		
4. COMPUTER SERVICES				0		
5. SUBAWARDS				25,535		
6. OTHER				0		
TOTAL OTHER DIRECT COSTS				32,535		
H. TOTAL DIRECT COSTS (A THROUGH G)				101,990		
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Direct Costs (Rate: 43.0000, Base: 76455)**						
TOTAL INDIRECT COSTS (F&A)				32,876		
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				134,866		
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0		
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 134,866	$	
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $					
PI/PD NAME **Mark Fishbein**				FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* **Lynda Tuck**				Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET

Cumulative

ORGANIZATION: Mississippi State University			FOR NSF USE ONLY		
			PROPOSAL NO.	DURATION (months) Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: Mark Fishbein			AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Mark Fishbein - Assistant Professor	0.00	0.00	3.00	$ 14,990	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	3.00	14,990	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (3) POST DOCTORAL ASSOCIATES	36.00	0.00	0.00	99,891	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (6) UNDERGRADUATE STUDENTS				13,111	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				127,992	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				29,500	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				157,492	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.) $ 6,000					
TOTAL EQUIPMENT				6,000	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				35,785	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				25,700	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,000	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				67,551	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				95,251	
H. TOTAL DIRECT COSTS (A THROUGH G)				294,528	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				102,398	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				396,926	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 396,926	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME	FOR NSF USE ONLY		
Mark Fishbein	INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME*	Date Checked	Date Of Rate Sheet	Initials - ORG
Lynda Tuck			

C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

Budget Justification

A. Senior Personnel

The PI is Dr. Mark Fishbein, who will oversee administration and direction of the project, participate in scent data collection and analysis, oversee and participate in the pollination experiments, and participate in the publication of the results. The PI is on a 9-month appointment as a faculty member at Mississippi State University. During the academic year, the PI will contribute to the project as part of his duties as a faculty member. Salary is requested for summer support (1.0 FTE) during the funding period at 1/9 of current annual salary ($43,216) in the first year of the study, with 4% cost-of-living increments in the second and third years.

B. Other Personnel

One postdoctoral research associate will carry out the collection and analysis of the scent data, cultivation and controlled crosses of experimental plants, and participate in the field experiments and the publication of the results. Ms. Tracey Slotta has been identified as an excellent candidate for this position. She has collected preliminary scent data, is familiar with the experimental plants, and is currently at student at Virginia Polytechnic Institute and State University, in close proximity to the field sites. The postdoctoral associate's salary will be $32,000 in the first year with 4% cost-of-living increments in the second and third years. Undergraduate students will participate in collection of scent data and pollination data and experimental crosses, under the direction of the PI and postdoctoral associate. Undergraduate students will be paid a stipend of $2100 for eight weeks of work in the first year with 4% cost-of-living increments in the second and third years

C. Fringe Benefits

For each year of the project, fringe benefits are calculated as 25.5% of the PI's + postdoctoral associate's salaries + 1% of the wages of undergraduate students for workman's compensation.

D. Equipment

A desktop computer (CPU, display, printer--$6,000) is requested for the use of the postdoctoral associate.

E. Travel

Funding is requested each year of the project for travel to field sites and laboratories. In year 1, travel will include 2 trips by the PI (and undergraduates) and postdoc to Shenandoah NP (scent collection and seed collection), 1 trip by the PI (and undergraduates) and postdoc to Blandy Experimental Farm (grow seeds of experimental plants), 1 trip by the postdoc to University of South Carolina (scent analysis), 1 trip by the postdoc to SUNY-Cortland (allozyme analysis), and 1 trip by the postdoc to Mississippi State University (data analysis), for a total of 8900 mi. and $3338 at $0.375/mi. The PI, undergraduates, and postdoc will camp while in the field. Funding for lodging is requested for the visits of the postdoc to USC, SUNY-Cortland, and MSU for 35 days at $40/day ($1400 total). Funding for food is requested for the same 35 days of travel for the postdoc, plus 46 person-field days for the PI, 2 undergraduates, and postdoc at $30/day for a total of $2430.

In year 2, travel will include 1 trip by the PI (and undergraduates) and postdoc to Blandy Experimental Farm (perform crosses and collect scents of experimental plants), 1 trip by the PI and postdoc to Blandy Experimental Farm (grow seeds of experimental plants), 1 trip by the PI (and undergraduates) and postdoc to Shenandoah NP (pollination experiments), 1 trip by the postdoc to Shenandoah NP (fruit set data and seed collection), 1 trip by the postdoc to University of South Carolina (scent analysis), and 1 trip by the postdoc to Mississippi State University (data analysis), for a total of 7050 mi. and $2644 at $0.375/mi. The PI, undergraduates, and postdoc will camp while in the field. Funding for lodging is requested for the visits of the postdoc to USC and MSU for 28 days at $40/day ($1120 total). Funding for food is requested for the same 28 days of travel for the postdoc, plus 95 person-field days for the PI, 2 undergraduates, and postdoc at $30/day for a total of $3690. Funding is requested for truck rental for transporting flowering plants from Blandy to Shenandoah NP ($250).

In year 3, travel will include 1 trip by the PI (and undergraduates) and postdoc to Blandy Experimental Farm (perform crosses and collect scents of experimental plants), 1 trip by the PI and postdoc to Blandy Experimental Farm (grow seeds of experimental plants), 1 trip by the PI (and undergraduates) and postdoc to Shenandoah NP (pollination experiments), 1 trip by the postdoc to Blandy Expermental Farm (collect seeds of experimental plants), 2 trips by the postdoc to University of South Carolina (scent analysis), and 2 trips by the postdoc to Mississippi State University (data analysis), for a total of 11,100 mi. and $4163 at $0.375/mi. The PI, undergraduates, and postdoc will camp while in the field. Funding for lodging is requested for the visits of the postdoc to USC and MSU for 56 days at $40/day ($2240 total). Funding for food is requested for the same 56 days of travel for the postdoc, plus 86 person-field days for the PI, 2 undergraduates, and postdoc at $30/day for a total of $4260. Funding is requested for truck rental for transporting flowering plants from Blandy to Shenandoah NP ($250).

Funding is also requested for travel to national meetings for the PI, postdoctoral associate, and each undergraduate student. The purpose of these trips is disseminate the results of the research and providing training to the students. Students will be expected to give oral or poster presentations. The cost for each participant is estimated at $1000, for a total of $2000 in the first year (PI and postdoc) and $4000 for all participants in each of the remaining years.

All proposed travel is considered domestic under the proposal guidelines.

F. Participant Support Costs

None.

G. Other Direct Costs

Supply costs in the first year include pumps for scent collection (10 at $600 each), $2000 expendable supplies for scent collection (including helium tanks and GC columns) and hand pollinations, $1000 for greenhouse supplies (pots, soil, pesticide, fertilizer), and $3000 for greenhouse utility charges/rental (heating, cooling and lighting).

Supply costs in the second year include $2000 expendable supplies for scent collection (including helium tanks and GC columns) and hand pollinations, $2000 expendable supplies for pollinator observations (including pollination bags, Plexiglas, flight cages, collection bottles, fixative), $700 for greenhouse supplies (pots, soil, pesticide, fertilizer), and $3000 for greenhouse utility charges/rental (heating, cooling and lighting). Commercial pollinators

(*Bombus impatiens*) will be provided free of charge by Koppert Biological Systems (see attached letter of support from Mr. John Wolf).

Supply costs in the third year include $2000 expendable supplies for scent collection (including helium tanks and GC columns) and hand pollinations $1000 for greenhouse supplies (pots, soil, pesticide, fertilizer), and $3000 for greenhouse utility charges/rental (heating, cooling and lighting).

A subcontract in the amount of $67,551 is made to Dr. Steven Broyles, SUNY-Cortland. Dr. Broyles and SUNY Cortland undergraduate research assistants will participate in several aspects of field and laboratory research of the proposed project. First, the SUNY Cortland research group will collect and process leaves, pollinia, and seeds for enzyme electrophoresis to be carried out in Dr. Broyles laboratory (Bowers 234) at SUNY Cortland. Second, the SUNY Cortland group will assist the PI in the collection of seeds, propagation of plants, and selection of plants for use in the experimental gardens and for scent analysis. Third, the SUNY Cortland research group will assist in fieldwork at Blandy Experimental Farm (Univ. of Virginia) and in Shenandoah National Park (VA). At these field sites, the SUNY Cortland research group will assist in mapping/marking of milkweed populations, collecting plant materials, conducting hand-pollinations, and conducting pollinator observations.

Publication costs are estimated at $500 per publication for journal page charges and reprints. Two publications are expected at the ends of the second and third years ($2000).

I. Indirect Costs

Indirect costs are calculated at 43% of total direct costs, minus the costs of the subcontract amount after the first year and equipment.

Yr 1 = .43* ($97,754 - $6,000) = $38,164
Yr 2 = .43* ($97,784 - $24,859) = $31,358
Yr 3 = .43* ($101,990- $25,535) = $32,876

SUMMARY PROPOSAL BUDGET YEAR 1

ORGANIZATION SUNY College at Cortland				FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Steven B Broyles				AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Steven B Broyles - Principal Investigator	0.00	0.00	1.00	$ 5,670	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	5,670	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (2) UNDERGRADUATE STUDENTS				2,500	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				8,170	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				1,039	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				9,209	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				860	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				1,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				1,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				11,069	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) On Campus MTDC (Rate: 55.0000, Base: 11069)					
TOTAL INDIRECT COSTS (F&A)				6,088	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				17,157	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 17,157	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME Steven B Broyles	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Glen Clarke	Date Checked	Date Of Rate Sheet	Initials - ORG

1 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

0415358

SUMMARY PROPOSAL BUDGET
YEAR 2

ORGANIZATION: SUNY College at Cortland				FOR NSF USE ONLY	
				PROPOSAL NO.	DURATION (months): Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: Steven B Broyles				AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Steven B Broyles - Principal Investigator	0.00	0.00	1.00	$ 5,840	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	5,840	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (3) UNDERGRADUATE STUDENTS				5,200	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				11,040	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				1,178	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				12,218	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				2,820	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				1,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				1,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				16,038	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) On Campus MTDC (Rate: 55.0000, Base: 16038)					
TOTAL INDIRECT COSTS (F&A)				8,821	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				24,859	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 24,859	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME: Steven B Broyles	FOR NSF USE ONLY		
	INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME*: Glen Clarke	Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

0415358

SUMMARY PROPOSAL BUDGET — YEAR 3

ORGANIZATION **SUNY College at Cortland**	FOR NSF USE ONLY PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR **Steven B Broyles**	AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. **Steven B Broyles - Principal Investigator**	0.00	0.00	1.00	$ 6,015	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	1.00	6,015	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (3) UNDERGRADUATE STUDENTS				5,200	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				11,215	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				1,239	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				12,454	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				2,820	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				1,200	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				1,200	
H. TOTAL DIRECT COSTS (A THROUGH G)				16,474	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **On Campus MTDC (Rate: 55.0000, Base: 16474)**					
TOTAL INDIRECT COSTS (F&A)				9,061	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				25,535	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 25,535	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI/PD NAME **Steven B Broyles**	FOR NSF USE ONLY INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* **Glen Clarke**	Date Checked	Date Of Rate Sheet	Initials - ORG

 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY

ORGANIZATION SUNY College at Cortland	PROPOSAL NO.	DURATION (months) Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Steven B Broyles	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Steven B Broyles - Principal Investigator	0.00	0.00	3.00	$ 17,525	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	3.00	17,525	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	0	
3. (0) GRADUATE STUDENTS				0	
4. (8) UNDERGRADUATE STUDENTS				12,900	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				30,425	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				3,456	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				33,881	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				6,500	
2. FOREIGN				0	
F. PARTICIPANT SUPPORT COSTS 1. STIPENDS $ 0 2. TRAVEL 0 3. SUBSISTENCE 0 4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				3,200	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				3,200	
H. TOTAL DIRECT COSTS (A THROUGH G)				43,581	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				23,970	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				67,551	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.C.6.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 67,551	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME	FOR NSF USE ONLY		
Steven B Broyles	INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME*	Date Checked	Date Of Rate Sheet	Initials - ORG
Glen Clarke			

C *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

Budget Justification

A. Senior Personnel (Total = $17,525)

One month of summer salary per year is requested for Dr. Broyles. Salary is based on 1/9 of the academic year. During this month, the PI will devote full effort to research. A 2.0 % salary increase is projected for the first year and 3.0% per year thereafter. ($5,670, $5,840 and $6,015).

B. Other Personnel (Total = $12,900)

During the summers of years 2 and 3, a biology undergraduate student will be hired to participate in the proposed research each summer. These students will be intimately involved in field and laboratory research which will include managing collections, field records, sample preparation, laboratory data collection, and data analysis. Students will be paid a stipend of $2,700 for eight weeks.

During each of the three academic years, one biology undergraduate will be hired each semester to assist with the enzyme extractions and gel runs. Students will be paid a ten week part-time stipend of $1250 (Total $2500 per year).

C. Fringe Benefits (Total = $3,456)

SUNY requests 17.0% for summer 2004 with planned increases of 0.5% per year the following years. ($964, $1,022, and $1,083). The College also requests 3.0% per year for student fringe benefits ($75; $156; $156).

D. Permanent Equipment

No permanent equipment over $5,000 is requested (see below).

E. Travel. (Total = $6,500)

I anticipate camping at Shenandoah National Park and utilizing Blandy Experimental Farm (University of Virginia). In the past, entrance fees to Shenandoah National Park have been waived when a collecting permit was obtained. Funds for travel to meetings in years 2 and 3 are also requested for the PI and one student ($1400 / year)

Year 1. Funds are requested for fourteen days of travel to Shenandoah National Park and Blacksburg, Virginia (800 mi. round trip) to collect leaf material and assist in hand-pollinations of *A. exaltata* and *A. syriaca*. Expenses for travel include $300 (800 mi x $0.375/mi) for mileage and $560 (14 days x $40/day) for lodging and food.

Year 2. Funds are requested for 14 days of travel to Shenandoah National Park. Work will be conducted on experimental plots and natural populations. Expenses for travel include $300 (800 mi x $0.375/mi) for mileage and $1120 (14 days x $40/day/individual x 2 individuals) for lodging and food.

Year 3. Funds are requested for seven days of travel to Shenandoah National Park (VA) and Blacksburg. Work will be conducted on hand pollination experiments. Expenses for travel include $300 (800 mi x $0.375/mi) for mileage and $1120 (14 days x $40/day/individual x 2 individuals) for lodging and food.

G. Other Direct Costs (Total = $3,200)

Materials and Supplies
Year 1. We anticipate completing allozyme analysis of 1000 samples (600 seedlings screened for artificial populations + 400 plants from Shenandoah National Park). The total cost of $1000 is based on estimates for purchasing reagents (1000 samples x 2 buffer systems/sample x 1gel/30 samples x $15/gel).

Year 2. We anticipate completing allozyme analysis on 1000 pollinia from experimental and natural populations. The total cost of $1000 is based on estimates for purchasing reagents (1000 samples x 2 buffer systems/sample x 1gel/30 samples x $15/gel).

Year 3. We anticipate completing allozyme analysis on 1200 pollinia from experimental and natural populations. The total cost of $1200 is based on estimates for purchasing reagents (1200 samples x 2 buffer systems/sample x 1gel/30 samples x $15/gel).

H. Total Direct Cost = $43,581

I. Indirect Costs =$23,970

SUNY indirect charges are 55% of modified total direct costs. The rate is established with the federal agency DHHS.

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Mark Fishbein**

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☒ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **REU: Conservation Biology in the Southeastern US**

Source of Support: **National Science Foundation**

Total Award Amount: $ **209,874** Total Award Period Covered: **04/01/03 - 03/31/06**

Location of Project: **Mississippi State University**

Person-Months Per Year Committed to the Project. Cal: **0.00** Acad: **0.00** Sumr: **0.50**

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Phylogenetic Systematics and Biogeography of Asclepias**

Source of Support: **National Science Foundation**

Total Award Amount: $ **327,062** Total Award Period Covered: **09/01/04 - 08/31/07**

Location of Project: **Mississippi State University**

Person-Months Per Year Committed to the Project. Cal: **0.00** Acad: **0.00** Sumr: **2.00**

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Summ:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: **Steven Broyles**

Other agencies (including NSF) to which this proposal has been/will be submitted.

Support: ☐ Current ☒ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title: **Floral Scents of Hybrids: Bridge or Barrier to Interspecific Gene Flow**

Source of Support: **NSF**

Total Award Amount: $ **67,551** Total Award Period Covered: **07/01/04 - 06/30/07**

Location of Project: **SUNY Cortland**

Person-Months Per Year Committed to the Project. Cal: **1.45** Acad: **0.45** Sumr: **1.00**

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

Support: ☐ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support

Project/Proposal Title:

Source of Support:

Total Award Amount: $ Total Award Period Covered:

Location of Project:

Person-Months Per Year Committed to the Project. Cal: Acad: Summ:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

FACILITIES, EQUIPMENT & OTHER RESOURCES

FACILITIES: Identify the facilities to be used at each performance site listed and, as appropriate, indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use "Other" to describe the facilities at any other performance sites listed and at sites for field studies. USE additional pages as necessary.

Laboratory: **Much of the research will be conducted in the field or in collaborating labs. Scent analyses will be conducted in the lab of Dr. Rob Raguso (University of South Carolina; see attached letter of support). Allozyme analyses will be conducted in the lab of co-PI Dr. Steven Broyles**

Clinical:

Animal:

Computer: **The PI has ample computing facilities for data analysis in the laboratory and in his office, including an iMac dedicated to gel image collection and storage, a Power Mac G4 dual 1.25 GHz with 2 GB RAM desktop computer dedicated to phylogenetic analysis, a Dell Dimension XP Pentium 4 desktop**

Office: **The PI has adequate personal office space to carry out duties related to this proposal. Office support provided by the department includes secretarial personnel, mail service, photocopiers, and general office supplies.**

Other:

MAJOR EQUIPMENT: List the most important items available for this project and, as appropriate identifying the location and pertinent capabilities of each.

OTHER RESOURCES: Provide any information describing the other resources available for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations.

Blandy Experimental Farm will provide greenhouse space and field plots.

LABORATORY FACILITIES (continued):

(SUNY-Cortland; see subcontract). Both of these researchers are well equipped for the proposed research. The PI?s laboratory is 1,200 sq. ft. and well equipped for molecular and morphological studies, including refrigerators, -20C and ?80C C freezers, 37C and cooling standard and shaking incubators, horizontal gel electrophoresis systems, two temperature gradient thermal cyclers, water baths, UV light source and digital gel documentation system, table top microcentrifuges and clinical centrifuge, dry heat blocks, and pH meter. An ice machine and autoclave are available in common use departmental facilities in the same wing of the biology building.

COMPUTER FACILITIES (continued):

computer, and a Power Mac G4 867 MHz desktop computer for general use. Laser printers are available. Networking is provided by Ethernet connections to laboratories and offices and supported by the university Information Technology Services department.

Facilities and Other Resources

Dr. Broyles personal laboratory is fully equipped to perform starch gel electrophoresis. The electrophoresis equipment includes gel and electrode apparatuses and power packs to run 8 gels. In addition, the lab is equipped with an analytical balance, UV light table, pH meter, drying oven and glassware. The Department of Biological Sciences has facilities to produce distilled water.

The Molecular Biology/Genetics laboratory is a multi-user facility. Cell Biology (BIO 210) and Genetics (BIO 312) frequently use lab space for class projects, as well as individual faculty for personal research (Drs. Baroni, Broyles, and Conklin) and student projects. Equipment in the laboratory includes an unrefrigerated microcentrifuge, MJ Research Thermocycler, autoclave, -20^{O}C freezer, microwave oven, pipets, water baths, power supplies, and gel apparatuses. In addition, the Department owns a -70^{O}C freezer.

Departmental greenhouse facilities are managed and maintained by a Departmental technician and work-study students. These facilities are sufficient for growing and maintaining milkweeds that may be used in the research.

Office facilities includes a computer, printer and internet capabilities.

The College supports numerous on-campus computer facilities. Two computer facilities with MacIntosh and Dell computers with printers are housed in Bowers Hall (Natural Science Building). The College purchases licenses to many software programs with include spreadsheet, word processing, and statistical packages.

7 January 2004

Dr. Mark Fishbein
Dept. of Biological Sciences
Mississippi State University
P.O. Box GY
Mississippi State, MS 39762 USA

Dear Mark,

I am eager to proceed with our planned collaboration on the use of floral scent characters to track pollinator-mediated introgression between *Asclepias syriaca* and *A. exaltata* in the Blue Ridge Mts. The pilot analyses conducted by Tracey Slotta while visiting my lab demonstrated that fragrance differences between these species are quite distinct, both in terms of odor intensity as well as chemical composition, and that our analytical methods are sufficiently sensitive to detect these differences from single umbels.

I am happy to provide the use of my laboratory at the University of South Carolina, including access to my Shimadzu QP5000 Gas Chromatograph-Mass Spectrometer, for the purpose of scent analysis in naturally occurring and hand-crossed hybrid plants. These resources include the appropriate analytical software, computer facilities, chemical standards and my own experience having analysed fragrance from over 100 species of flowering plants and fungi. I look forward to a stimulating and productive collaboration.

Sincerely,

Robert A. Raguso
Assistant Professor
Dept. of Biological Sciences
University of South Carolina
Columbia SC 29208 USA
raguso@biol.sc.edu

KOPPERT
BIOLOGICAL SYSTEMS

January 7, 2004

Mark Fishbein
Mississippi State University

Dear Mark,

Koppert Biological Systems, Inc. will provide to you 2 QUADS (8 bumblebee hives) at no cost for your milkweed hybridization project. However, we will require that you pay the shipping costs (approx. $60-$50).

Just let me know when you need the hives. We should be able to get them to you within 2 day. We of course would appreciate as much advance notice as possible for our planning purposes.

Regards

John Wolf
Technical Advisor
Koppert Biological Systems, Inc.
Romulus, MI 48174
(800) 928-8827

Blandy Experimental Farm
The State Arboretum of Virginia

University of Virginia

8 January 2004

Dr. Mark Fishbein
Department of Biological Sciences
Mississippi State University

Dr. Fishbein,

The University of Virginia's Blandy Experimental Farm will be able to provide the necessary greenhouse, field, and lab space for your experiments on hybridization in *Asclepias*. We can also provide dorm space for you, Dr. Broyles, and your field crew. We look forward to your becoming part of the research community here. Good luck with your proposal.

Sincerely,

David E. Carr
Assistant Professor
Environmental Sciences

400 Blandy Farm Lane • Boyce Virginia 22620
Phone: 540-837-1758 • Fax: 540-837-1523 • http://www.virginia.edu/~blandy
On Route 50 in Clarke County, between Route 340 and the Shenandoah River.

THE RESEARCH FOUNDATION
The State University of New York

State University College at Cortland

Research Administration
Sponsored Programs

Amy Henderson-Harr
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PO Box 2000
402 Miller Building
Cortland, New York 13045

(607) 753-2511
Fax (607) 753-5590

January 8, 2004

RE: Local File #181

To Whom It May Concern:

The State University of New York College at Cortland (SUNY Cortland) strongly supports the single proposal Collaborative Proposal of Principal Investigator Dr. Mark Fishbein at Mississippi State University entitled *"Floral Scents of Hybrids: Bridge or Barrier to Interspecific Gene Flow?"*. The proposal includes a planned sub-award from Mississippi State University to the Research Foundation of SUNY on behalf of and in conjunction with SUNY Cortland.

SUNY Cortland requests $67,551 during the period of performance of July 1, 2004 through June 30, 2007 for its proposed collaborative activities. The Principal Investigator under the sub-award is Dr. Steven Broyles, Biological Sciences, SUNY Cortland.

The Research Foundation of the State University of New York serves as fiscal administrator of the State University of New York College at Cortland awards. As such, award notification or inquiries should be directed to Ms. Mary Riley, Research Foundation of State University of New York, P.O. Box 9, Albany, NY 12201-0009. Ms. Riley may be reached at (518) 434-7105 or by e-mail at mary.riley@rfsuny.org. Pre-award inquiries should be directed to my attention at (607) 753-2511 or by e-mail at glenc@cortland.edu.

We hope that this proposal meets with your most favorable response. Thank you for your consideration.

Sincerely,

Glen C. Clarke

Glen C. Clarke
Assistant Director

GCC/pas

pc: S. Broyles
M. Fishbein
P. Catterfeld